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Studies on Blood and Plasma Catalase

The 3rd Report

The Blood and Plasma Catalase Content in Blood Diseases

By

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From our former experiments[1], it is concluded that the blood catalase content changes for itself in spite of the close connection with erythrocytes and hemoglobin. But the function of the plasma catalase is still unknown.

Therefore, it is important to clarify the clinical significance of the blood and plasma catalase content in patients of diseases of the blood.

Experimental

Results

1. Anemia caused by cancers

The results obtained from 6 cases of anemia caused by cancers show that the blood and plasma catalase content, catalase index and hemoglobin-catalase index decrease more than in healthy persons and tend to be notable when the process of cancer degenerates (Table I). On the contrary, the plasma-blood catalase ratio increases more than in healthy persons and it seems to decrease when cancers are in advanced stage.

Therefore, it is presumed that the decrease of the blood catalase in anemia patients caused by cancers is not only more pronounced than that of the plasma catalase, but also the catalase content in each red corpuscle decreases remarkably.

2. Anemia caused by ulcers

Data presented in Table I show that the blood catalase content decreases noticeably in anemia patients caused by ulcers, while the plasma catalase content, catalase index, hemoglobin-catalase index lie within a normal value. On the contrary, the plasma-blood catalase ratio increases more markedly than that of healthy persons and seems to decrease when ulcer is cured.

Thus it is concluded that the decrease of the blood catalase is accompanied by the decrease of erythrocytes and hemoglobin.

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TABLE I

Anemias Caused by Cancers and Ulcers

Name	Diagnosis	Date	Erythrocytes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-catalase index	Plasma-blood catalase ratio	Remarks
Y.R.	Gastric cancer	18/VII	207	28	0.68	2.99	12.58	1.44	0.62	4.21	2/VIII
		1/VIII	200	25	0.62	2.92	12.24	1.46	0.68	4.18	op.
M.U.	ditto, Peritonitis	12/V	139	18	0.65	3.13	12.58	2.25	1.01	4.02	17/VI
		5/VI	250	32	0.64	3.77	10.88	1.51	0.68	2.89	+
S.M.	ditto, Hepatic cancer	14/V	243	25	0.52	4.32	11.22	1.77	1.00	2.60	9/VIII
		20/VI	235	28	0.60	3.43	9.86	1.46	0.71	2.87	+
		23/VII	244	27	0.55	2.21	9.18	0.91	0.48	4.16	
O.Y.	Gastric cancer	17/I	153	39	1.27	4.42	12.24	2.28	0.66	2.77	
		6/II	181	42	1.16	5.51	10.20	3.04	0.76	1.83	
I.C.	ditto	30/IV	241	39	0.81	4.44	10.20	1.86	0.67	2.27	24/V
		14/V	226	38	0.84	4.28	9.52	1.89	0.65	2.22	+
K.S.	Cancer of pancreas	14/V	193	45	1.16	5.07	10.54	2.63	0.65	2.08	12/VII
		6/VI	171	45	1.32	4.76	9.52	2.78	0.62	2.00	+
S.T.	Gastric ulcer	16/III	203	42	1.04	2.93	15.98	1.44	0.41	5.45	transfusion
		5/IV	324	56	0.87	7.51	16.66	2.32	0.78	2.21	3 times
O.G.	ditto	16/VIII	214	50	1.17	3.81	14.96	1.78	0.44	3.92	
		15/IX	330	62	0.94	7.48	13.94	2.26	0.70	1.87	
K.O.	ditto	17/I	175	35	1.00	5.71	13.94	3.26	0.95	2.44	ditto
		7/II	271	65	1.20	8.50	14.28	3.13	0.76	1.68	7 times
Y.J.	ditto	8/IV	230	38	0.83	7.68	15.30	3.34	1.18	1.89	ditto
		5/V	397	70	0.88	8.30	14.28	2.09	0.69	1.72	4 times

3. Pernicious anemia

As to pernicious anemia patient we found that the blood catalase content decreased and the catalase index and plasma-blood catalase ratio increased in marked degree, while the plasma catalase content and hemoglobin-catalase index were almost normal (Table II). When patient was recovered with increased erythrocytes, the blood catalase increased and the catalase index and plasma-blood catalase ratio decreased to a normal value.

Therefore, it appears in the case of pernicious anemia that the catalase content in each red corpuscle increases in company with hemoglobin, while the whole quantity of the blood catalase decreases pronouncedly.

4. Aplastic anemia and other anemias

The results obtained from a case of aplastic anemia show that the blood and plasma catalase content decrease remarkably, but the catalase index and hemoglobin-catalase index to a slight degree, while the plasma-

TABLE II

Various Anemias

Name	Diagnosis	Date	Erythrocytes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-catalase index	Plasma-blood catalase ratio	Remarks
W.T.	Pernicious anemia	11/VI	69	27	1.96	4.49	12.92	6.50	0.97	2.87	A slight recovery by transfusion.
		19/VI	68	25	1.84	4.49	19.04	6.60	1.04	4.24	
		3/VII	134	37	1.38	4.45	14.28	3.32	0.70	3.21	
		13/VII	122	35	1.43	3.43	13.94	2.81	0.57	4.07	
		23/VII	135	46	1.70	6.60	10.88	4.88	0.83	1.65	
		10/VIII	154	51	1.65	6.09	8.16	3.95	0.69	1.34	
		30/VIII	239	55	1.14	6.80	13.94	2.84	0.72	2.05	
I.M.	Aplastic anemia	29/V	80	16	1.00	1.73	6.80	2.17	0.64	3.92	Transfusions were of no effect.
		18/VI	112	20	0.89	2.48	6.80	2.22	0.72	2.73	
		2/VII	152	28	0.92	2.41	5.78	1.58	0.50	2.39	
		23/VII	140	29	1.03	2.82	6.46	2.01	0.57	2.29	
T.N.	Thrombocytopenic purpura	23/IV	141	20	0.71	3.14	7.48	2.25	0.91	2.39	Thrombocytes 23/IV 7000 4/V 0
		4/V	103	20	0.95	3.26	4.74	3.17	0.95	1.45	
S.M.	ditto	5/VIII	85	20	1.17	2.18	6.80	2.55	0.63	3.13	ditto 5/VIII 5000
		12/VIII	53	12	1.13	1.40	6.12	2.64	0.68	4.37	

blood catalase ratio tends to increase (Table II).

Therefore, the decrease of the blood catalase in the case of aplastic anemia is more remarkable than that of the plasma catalase and the catalase content in each red corpuscle decreases also in a slight degree.

As to thrombocytopenic purpura, whose anemia resembles closely to aplastic anemia, the results are generally the same as in aplastic anemia.

By the by, the fact that the plasma catalase was found from the blood sample of T.N. who had almost no thrombocytes, suggests that a part of the plasma catalase does not originate in thrombocytes.

Table III shows that the blood catalase of a paroxysmal hemoglobinuria patient makes a marked decrease, but the plasma catalase, catalase index, hemoglobin-catalase index lie within a normal value, while the plasma-blood catalase ratio increases and returns to a normal value when the process takes a favourable turn. In the case of an artificial hemolysis in a living body by Rosenbach's cooling test, the plsama catalase and plasma-blood catalase ratio show a remarkable increase in spite of the diminution of erythrocytes, hemoglobin and blood catalase without any change of the catalase index and hemoglobin-catalase index.

Therefore, such hemolysis in a living body seems to be significant as to the origin of the plasma catalase.

5. Leukemia

Our results obtained from chronic myelogenous leukemia show, as

TABLE III

Rosenbach's Cooling Test of Paroxysmal Hemoglobinuria

Date	Time	Erythrocytes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-catalase index	Plasma-blood catalase ratio	Urine		
										Colour	Albumin	Occult blood
15/II	Before test	290	75	1.29	6.97	14.28	2.40	0.54	2.25	red-brown	+	−
	15 min. after	304	72	1.18	8.36	28.56	2.75	0.67	3.41	ditto	+	−
	30 min. »	332	75	1.13	7.07	19.04	2.13	0.54	2.69	black-brown	++	+
	1 hour »	271	65	1.20	6.77	54.40	2.49	0.60	8.03	ditto	++	++
	2 hours »	274	63	1.14	6.97	88.40	2.54	0.64	12.68	ditto	++	++
	3 hours »	307	61	0.93	7.28	40.80	2.36	0.69	5.61	ditto	++	++
	4 hours »	305	65	1.06	7.38	21.76	2.42	0.66	2.79	ditto	++	++
	5 hours »	311	66	1.06	7.92	45.56	2.55	0.70	5.75	ditto	+	±
20/V	Before test	440	81	0.92	8.50	10.88	1.93	0.61	1.28	yellow	−	−
	15 min. after	437	73	0.84	9.04	27.20	2.06	0.72	3.01	ditto	−	−
	30 min. »	388	79	1.03	8.23	14.28	2.12	0.61	1.74	ditto	+	±
	1 hour »	436	80	0.92	8.23	25.84	1.89	0.60	3.14	black-brown	++	++
	2 hours »	381	78	1.02	7.79	42.84	2.04	0.58	5.50	red-brown	++	+
	3 hours »	418	75	0.90	7.92	34.00	1.90	0.61	4.30	ditto	+	±
	4 hours »	364	65	0.89	8.84	44.20	2.43	0.79	5.00	ditto	+	−

presented in Table IV, that the blood catalase content, catalase index, hemoglobin-catalase index decrease more or less in an early stage, but return to a normal value, as the condition of this disease is recovered by radiological treatment. When 0.7 cc. adrenalin chloride is subcutaneously injected in order to raise the number of leucocytes in the peripheric blood, we found a slight increase of the plasma catalase content and plasma-blood catalase ratio, while the blood catalase content, catalase index, hemoglobin-catalase index are almost unchangeable.

Therefore, the contraction of the spleen after injecting adrenalin has influence on the increase of the plasma catalase.

Contrary to chronic myelogenous leukemia, data obtained from acute myelogenous leukemia show, as presented in Table IV, that the blood catalase content decreases, being accompanied with marked anemia, but the plasma catalase content and plasma-blood catalase raito increase remarkably.

6. Agranulocytosis

In a case of agranulocytosis caused by injecting salvarsan for the treatment of hereditary syphilis, we found the increase of the plasma catalase content and plasma-blood catalase ratio, while the blood catalase content, catalase index and hemoglobin-catalase index lie within a normal value (TableV).

Therefore, it is presumed that there is no close connection between the blood catalase content and the number of leucocytes, and the plasma catalase content is influenced also by another factor in addition to the

TABLE IV

Adrenalin Test of Leukemia

Name	Diagnosis	Date	Time	Erythro-cytes	Leucocy-tes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-cata-lase index	Plasma-blood cata-lase ratio
K.R.	Chronic myelogenous leukemia	12/II	Before test	426	76500	78	0.92	7.99		1.88	0.86	
			15 min. after	457	193200	80	0.88	7.75		1.69	0.56	
			30 min. »	414	199300	78	0.94	8.13		1.96	0.61	
			45 min. »	456	271400	80	0.88	8.40		1.86	0.61	
			1 hour »	460	255900	78	0.85	8.13		1.77	0.61	
			1. 5 hours»	400	204400	79	0.99	7.72		1.93	0.57	
			2 hours »	390	176000	72	0.92	7.79		1.99	0.63	
		18/IV	Before test	531	30400	100	0.94	11.70	17.00	2.20	0.68	1.45
			15 min. after	520	22400	102	0.97	11.70	26.18	2.24	0.67	2.23
			30 min. »	587	37000	117	0.98	11.73	23.12	2.03	0.59	1.96
			45 min. »	552	47400	118	1.07	11.53	23.12	2.09	0.58	2.00
			1 hour »	584	43200	100	0.86	11.63	12.92	1.99	0.68	1.11
			1. 5 hours»	536	38400	102	0.95	11.46	16.66	2.14	0.66	1.45
			2 hours »	558	36800	95	0.85	11.73	20.74	2.10	0.72	1.76
I.S.	ditto	10/IX		300	16900	51	0.85	5.98	16.66	1.99	0.68	2.78
		28/IX		247	6400	45	0.91	5.10	15.64	2.06	0.66	3.07
I.T.	Acute myelogenous leukemia	14/VII	Before test	133	33200	41	1.54	3.50	23.80	2.63	0.49	6.78
			15 min. after	143	38200	40	1.41	3.98	17.00	2.78	0.58	4.28
			30 min. »	151	43800	40	1.33	4.08	23.80	2.70	0.59	5.83
			45 min. »	147	37600	39	1.33	3.40	9.52	2.31	0.51	2.80
			1 hour »	137	34200	37	1.35	3.60	10.20	2.62	0.56	2.83
			1. 5 hours»	143	28600	37	1.29	3.33	8.84	2.33	0.52	2.66
			2 hours »	140	28000	41	1.41	3.43	6.80	2.45	0.48	1.98

TABLE V

Agranulocytosis

Date	Erythro-cytes	Leuco-cytes	Hb.	Hb-index	Blood catalase	Plasma catalase	Catalase index	Hb-catalase index	Plasma-blood catalase ratio
25/V	539	1150	104	0.97	11.55	40.80	2.05	0.62	3.69
27/V	540	800	104	0.96	10.08	37.40	2.02	0.61	3.43
29/V	566	1150	106	0.94	10.85	36.04	1.92	0.60	3.32
8/VI	510	5600	100	0.98	11.39	32.64	2.22	0.66	2.86
18/VI	499	2800	88	0.98	11.97	25.16	2.39	0.85	2.29
3/VII	435	4200	98	1.13	10.09	19.72	2.32	0.60	1.95
14/VII	510	6500	105	1.03	11.12	21.42	2.18	0.61	1.93

hemolysis and the function of the spleen.

DISCUSSION

Many workers accept the decrease of the blood catalase content in pernicious anemia patient, and v. Thienen[2] reports also the decreased content of blood catalase in the cases of aplastic anemia, but Nakanishi[3]

asserts that the blood catalase content of paroxysmal hemoglobinuria is unchangeable. According to our former experiments[1], it is clear that the blood catalase content changes for itself in spite of the close connection with erythrocytes and hemoglobin, and then decreases to a certain degree in the cases of cancers and ulcers. The results obtained from these experiments show that the blood catalase content decreases in various anemia patients, especially in the cases of aplastic anemia, thrombocytopenic purpura and acute myelogenous leukemia.

As for the catalase index many investigators differ in opinion in the cases of pernicious anemia. v. Thienen[2], Strauss and Rammelt[4], Nissen[5], Zerner[6] and Nakanishi[3] recognize the increase of the catalase index, but Krumbhaar and Musser[7], Segall and Händel[8] deny such increase of the catalase index. Moreover, the catalase index of aplastic anemia is reported as normal by v. Thienen[2]. Many workers such as v. Thienen[2], Neumann[9], Strauss and Rammelt[4], Segall and Händel[8] report that the catalase index of chronic myelogenous leukemia falls in general within a normal value, while Zerner[6] and Nakanishi[3] recognize its increase. Data presented in these experiments show that the catalase index remains within a normal value in the cases of aplastic anemia, thrombocytopenic purpura, acute myelogenous leukemia, but that in anemia caused by ulcers and chronic myelogenous leukemia shows a slight decrease in early stage and returns to a normal value when the condition of these patients is recovered.

Contrary to anemia caused by cancers whose catalase index decreases markedly, the catalase index of pernicious anemia shows a remarkable increase in the worst stage, but returns rapidly to a normal value in the convalescence.

Because the hemoglobin-catalase index lies generally within a normal value except various anemias and acute myelogenous leukemia, the blood catalase content changes in the close connection with hemoglobin.

As for the plasma catalase content, Takisawa[10] reports that it decreases in cancer patients, but remains within a normal extent in ulcer sufferers. The results obtained from our experiments show that the plasma catalase content of anemia caused by ulcers, pernicious anemia and chronic myelogenous leukemia lies within a normal degree, but it decreases in the cases of anemia caused by cancers, aplastic anemia and thrombocytopenic purpura, while that of actue myelogenous leukemia and agranulocytosis increases. Especially in the latter case, the plasma catalase content shows an evident elevation in early stage and returns to a normal value when the condition of the patient is recovered. Moreover, we found the increased content of plasma catalase when the hemolysis in a living body was induced by Rosenbach's cooling test in a case of

paroxysmal hemoglobinuria, or the spleen was contracted by adrenalin injection in a case of chronic myelogenous leukemia.

Therefore, it is presumed that the plasma catalase content is influenced to a great extent not only by the hemopoietic function, but also by the hemolysis in a living body and the function of the spleen.

Summary

From these experiments it can be concluded as follows: the blood catalase content changes in the close connection with erythrocytes and hemoglobin, but the plasma catalase content is influenced to a great extent not only by the hemopoietic function, but also by the hemolysis in a living body and the function of the spleen.

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On the Chemical Properties of the Z-Granules

II. Hydrolysis Test

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Imprints of rabbit bone marrow fixed in susa solution were incubated in 1/10 *N* HCl for some duration at 57°C, washed in the water and then stained with the aqueous solution of thionin (0.2%) and sodium acetate (5%).[1] The Z-granules[1][2] characteristic to the granulocyte series (which are stained metachromatically yellow, reddish yellow to brown with the dye when processed without prior hydrolysis) lost their metachromasia almost completely by 40 minutes incubation and remained as stained blue violet. At the same time it was noticed that the chromatin substance, especially in the erythroblast series, becomes partly to show brown metachromasia which was, however, lost when hydrolysed in advance.

On the other hand, we became aware that the hydrolysed desoxyribonucleic acid (DNA) reveals similar metachromasia. 0.1 g sodium salt of thymo-DNA was dissolved in 16 cc. of 1/10 *N* HCl and heated in the boiling water for 3 hours. On addition of a small amount of the thionin-sodium acetate solution to a drop of the hydrolysed DNA solution on an objectglass, there precipitated immediately DNA granules. The specimen was dried and observed under oil immersion. All granules were found metachromatically stained reddish brown to brown.

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Cone-and Rod-Processes in Peripheral Color Vision

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Introduction

Methods used in researches in vision generally consist in measuring either a response to a stimulus of given energy or the energy required to produce a threshold response (or any other constant response). In any case, it seems difficult to isolate either from a mixture of cone- and rod-responses to a given superthreshold stimulus applied to a retinal region where photopic and scotopic receptors co-exist. Although the microelectrode technique of Granit[1-2] enables us to separate one from the other mechanically, a simultaneous record of cone- and rod-responses is difficult to obtain by this method.

Motokawa & Ebe[3] and Motokawa *et al*[4] have recently provided a method for separating the scotopic process from the photopic, based upon the difference in time course of electrical sensitivity following a brief illumination. Utilizing this method I attempted in the present investigation to study the behavior of cones and rods with special reference to retinal location.

Experimental

Method

In the method mentioned above, the electrical supernormality of the eye after exposure to light is taken as a measure for the effect of the light used. The details of the apparatus and procedure to determine electrical sensitivity are given in Motokawa's papers.[5-7]

After a preliminary dark adaptation of about 20 minutes, the eye was exposed to colored light for 0.5 sec., and stimulated with a constant current pulse of 100 msec. in duration at different times after termination of the light stimulus. The index of excitation was the least perceptible electrical phosphene. The electrodes were placed on the forehead slightly

above the eyebrow and on the homolateral temple. The electrical threshold was measured in such a way that the stimulating voltage was reduced from a sufficiently high level step by step until the subject could no more distinguish an electrical phosphene from the background of intrinsic light of the retina. Near the threshold the effect of each stimulus was compared with that of a control stimulus, i.e. one far below the threshold.[7] To determine one threshold value it was necessary to repeat such a trial from 20 to 40 times. The reference threshold or threshold without pre-illumination was determined to calculate the degree of supernormality after the formula: $\zeta=100\ (E—E_{\circ})/E_{\circ}$, where E and $E_{\circ}$ denote electrical sensitivities (reciprocal of thresholds) determined with and without pre-illumination. Similar measurements had to be done at different times, for example 0.5, 1, 1.5, 2, 2.5 sec., etc. after the end of illumination to trace the time course of supernormality, in which the characteristic of each receptor manifests itself. The accuracy of measurements was such that a difference greater than 2 in terms of ζ was generally significant.

A circular patch of ground glass 2° in visual angle was used, upon which light from a spectroscope was projected. The spectrum used in this experiment was an equal-energy spectrum, whose intensity was 70 times as high as the threshold intensity for the red light of 650 mμ at the dark-adapted fovea. The energy was made equal by adjusting the width of the collimator slit of the spectroscope to the prescribed values computed from the known spectral energy distribution of the light source.

In order to vary systematically the retinal location illuminated, a fixation mark, a faint red light, was displaced along the horizontal meridian of the temporal field of the left eye.

Results

Retinal responses following an illumination are expressed in form of ζ-time curves or excitability curves in Fig. 1. The duration of the illumination was 0.5 sec. for the continuous curves and 4 sec. for the broken curves. They were obtained with red (650 mμ), yellow (585 mμ), green (530 mμ) and blue (470 mμ) lights at 20° from the center of the fovea. The broken curves generally consist of a single maximum, while the continuous ones consist of 2 elevations. The preceding elevation has its crest at about 1 sec. for the red light, at about 1.5 sec. for the yellow light, at about 2 sec. for the green light and at about 3 sec. for the blue light. In this manner the crest time of the preceding elevation depends upon the wave-length of the pre-illuminating light. On the contrary, the succeeding elevation of each curve shows a maximum at about 4 sec. irrespective of the wave-length of the pre-illuminating light. According to the analysis by Motokawa *et al*[3-4], this elevation independent of the

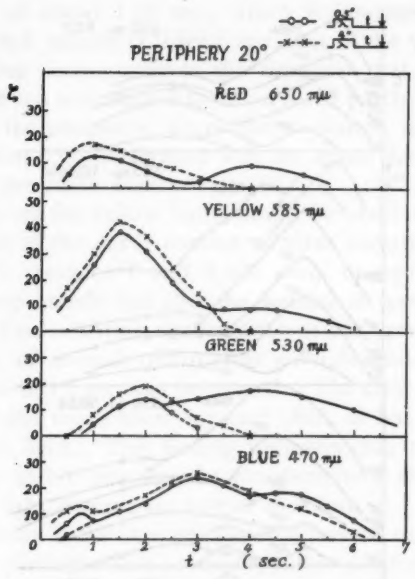

Fig. 1. Excitability curves obtained at periphery 20° with pre-illumination of 0.5 sec. (continuous curves) and 4 sec. (broken curves). Ordinates: percentage increases of electrical excitability above resting level. Abscissas: time in sec. after end of pre-illumination.

wave-length represents the scotopic process. This process orginates in the on-elements and shows its crest 4.5 seconds after the onset of illumination. The fact that the crest time of the succeeding elevation is about 4 sec. when measured from the end of 0.5 sec. illumination, corresponds to the property of the scotopic process described by Motokawa *et al.* When the illumination is too long, the scotopic process is lost sight of, because the process would come to an end during illumination. This is the reason why no succeeding elevation appeared in the case of illumination lasting for 4 sec. as can be seen in the broken curves.

Therefore the broken curves in Fig. 1 represent photopic processes unaccompanied by the scotopic. The fact that each broken curve is somewhat higher than the photopic part of the corresponding continuous curve may be ascribed to the effect of longer duration of illumination, for it is known that an increase in duration acts in the same way upon the magnitude of ζ as an increase in strength of illumination.[8]

The duration of illumination was made 0.5 sec. throughout the following experiment so as to make the photopic as well as the scotopic response equally visible, for it is the object of my investigation to throw

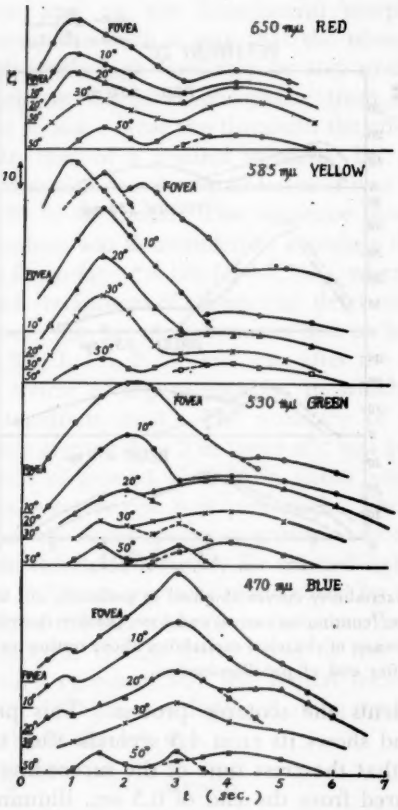

Fig. 2. Excitability curves obtained at various parts of retina. Figure by each curve indicates retinal location. Zero-level of each curve is given on left side.

light on the dual mechanism of the retina.

Cone- and rod-responses to red, yellow, green and blue lights are represented in Fig. 2. They were obtained at the fovea, 10°, 20°, 30° and 50° from the fovea. To begin with, let us observe the set of curves for red light. Although it is said that red light is little effective to stimulate rods, there can be seen fairly remarkable development of the scotopic process characterized by the longest crest time. The photopic process has its crest at about 1 sec. at the fovea and in the midperiphery, but its crest begins to shift toward a longer time at 30°. It is to be noted that the patch begins to lose its proper color in this region of the retina at the intensity used in this experiment. The photopic curve obtained at 30°

has a crest time of about 1.25 sec., which is intermediate between the crest time of the red process (1.0 sec.) and that of the yellow process (1.5 sec.). This finding corresponds to the situation that the patch looked orange at 30°. It is a well known fact that a red patch, when moved from the fovea toward the periphery, alters first to orange, then to yellow until it becomes colorless.[9-14] Reference will be made later to the complicated pattern of the curve obtained at 50°.

Next, the curves for yellow light should be observed. At the fovea, the photopic part of the curve consists of three elevations, among which those having their crests at 1 and 2 sec. seem to represent the red and green processes respectively and the one having its crest at 1.5 sec. the yellow process. This is further confirmation of our previous observation[15] that at the fovea of normal trichromats an independent yellow process having a crest time of 1.5 sec. can be proved. The crest times of the curves obtained outside the fovea show no such shift as has been observed in the curves for red light. This finding indicates that the yellow process is one of the most stable processes in the periphery of the retina. The curves for green light deserve no special mention.

The blue process is the slowest photopic process so that in the curves for blue light the photopic blue process is rather difficult to separate from the scotopic process. The initial small elevation of the blue curve is due to excitation of the red receptor.

The curves obtained at 50° are all alike and rather flat in shape. This property corresponds to a sensation of gray common to all colored lights falling on such a far periphery. As to the irregularities found on these curves, further experiments have been done, because they exceed the limit of our experimental errors. The data obtained and their physiological significance will, however, be reported in the next paper.

In Fig. 3A the maximal ζ-value of each photopic process shown in Fig. 2 is represented as a function of distance from the fovea.

Similarly the spatial distribution of the magnitude of the scotopic process (maximal ζ-value) caused by each colored light is shown in Fig. 3B. These figures indicate that the magnitudes of the yellow (Y) and the blue process (B) are greater than those of the green (G) and the red (R) process outside the fovea, and that green and blue lights are more effective to evoke the scotopic process than yellow and red lights. It is also worth noticing that the rod-response to each colored light is greatest at 20°. These findings fit in with the known properties of rods that they are more sensitive to lights of shorter wave-lengths than to those of longer wave-lengths and that the number of rods per unit area of the retina is largest at about 20° from the center.[16]

Some rod-responses seen in the curves of the fovea may be due to

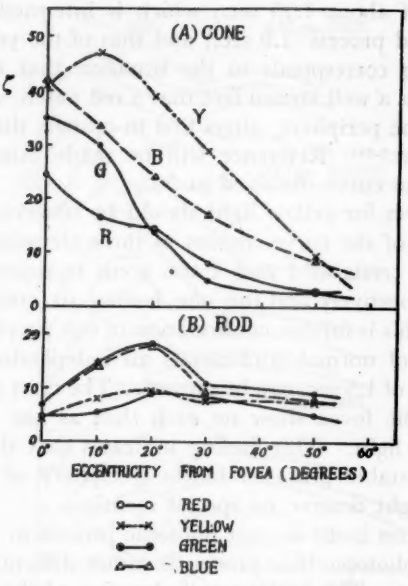

Fig. 3. (A) Response-magnitudes of photopic receptors with increasing distance from fovea (temporal horizontal meridian of left retina). R, Y, G and B represent responses to red (650 mμ), yellow (585 mμ), green (530 mμ) and blue (470 mμ) lights respectively. (B) Similar data for scotopic receptor.

the excitation of rods contained in the test-field of 2° in visual angle[16-18] or due to the excitation of extrafoveal rods caused by insufficient fixation of the eye.

The question now arises how the rod-responses to colored light modify the sensation of color. It is well known that peripheral color sensations become more desaturated as the intensity of colored light or retinal location is so changed that the excitation of rods is favored against that of cones. This desaturating effect of the rods must reduce the chromatic sensitivity of the peripheral retina.

Wentworth[18] measured the color-sensitivity of the peripheral retina and represented the reciprocal of the color-threshold as a function of distance from the fovea as illustrated in Fig. 4B. The spatial distribution curves for cone-responses shown in Fig. 3A show no satisfactory agreement with the distribution curves for color-sensitivity by Wentworth. The discrepancy may be due to the effect of the simultaneous excitation of the rods. So it is needed to make some correction for this rod-effect. It is, however, unknown to us how to correct the cone data. I made

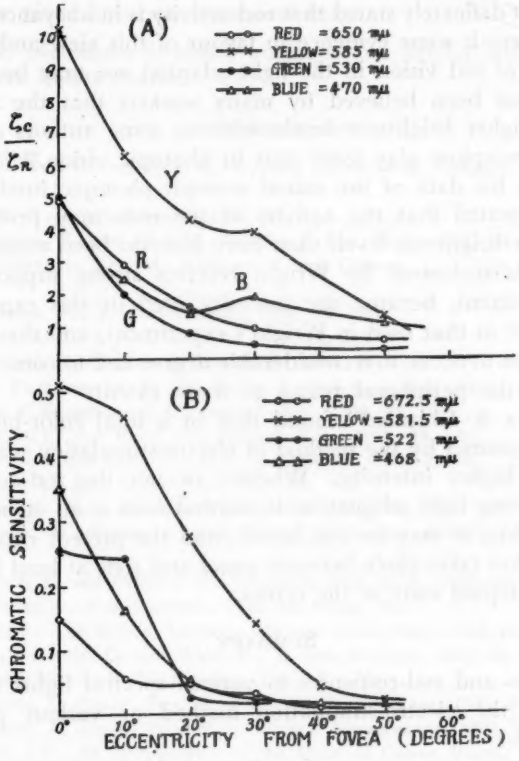

Fig. 4. (A) The ratio of cone-response to rod-response; cone-response in Fig. 3 A was divided by corresponding rod-response in Fig. 3 B. (B) Chromatic sensitivity curves of light-adapted eye obtained by Wentworth.

tentatively two kinds of correction, namely: 1. each rod-response was subtracted from the corresponding cone-response and 2. each cone-response was divided by the corresponding rod-response. It was found that the second correction was more suitable, because the curves representing the ratio of the cone-response to the rod-response could reproduce more closely the curves for color-sensitivity obtained by Wentworth as is shown in Fig. 4A.

Discussion

The original idea of the duplicity theory proposed by Max Schultze[19] and later established by v. Kries[20-21] appeared to design the visual mechanism to be exclusively dual. Parsons[22] stated, "In the duplicity

theory it is not definetely stated that rod activity is in abeyance in photopic vision, but there is some evidence in favour of this view and in any case the influence of rod vision in the light adapted eye may be regarded as slight." It has been believed by many workers that the rods do not function at higher brightness levels, whereas some authors claimed that the scotopic receptors play some part in photopic vision.[23-27]

Based on his data of the mixed scotopic-photopic luminosity curve Wright[28] suggested that the activity of the rods may probably persist even at higher brightness levels than have hitherto been assumed by some workers. This statement by Wright receives strong support from the present experiment, because the intensity used in this experiment was the same order as that used in Wright's experiment, and the rod-response has been shown to occur to a considerable degree and to concern the color-sensitivity of the peripheral retina to some extent.

Yonemura & Ishizaka[29] found that in a total color-blind the rod-activity as measured by the method of electro-stimulation was completely depressed at higher intensity. Whether or not the rod-activity is depressed by strong light adaptation in normal man is an open question.

At any rate, it may be concluded from the present experiment that some interaction takes place between cones and rods at least in the moderately light-adapted state of the retina.

Summary

The cone- and rod-responses to various spectral lights were studied by means of the electro-stimulation method at various parts of the human retina.

1. The cone-curves were found much higher than the rod-curves in every case.

2. The cone-curves for red light showed a maximum at 1 sec. in the central parts of the retina, but its crest began to shift toward a longer time at 30° from the fovea. It was pointed out that the shift was closely connected with the alteration of hue from red to orange.

No such shift could be observed in the curves for yellow and blue, whose maxima were always located at 1.5 and 3 sec. respectively.

3. The magnitude of the rod-response was greatest at the 20° periphery, where the population density of rods was greatest.

4. The response magnitudes of the photopic receptors were represented as a function of distance from the fovea and compared with the distribution curves for color-sensitivity by Wentworth. Agreement was not satisfactory, but the curves representing the ratio of the cone-response to the rod-response showed more satisfactory agreement with Wentworth's color-sensitivity curves. This fact suggests that some inter-

action takes place between cones and rods.

Prof. K. Motokawa furnished guidance and helpful criticism, for which I express here hearty thanks.

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On the Z-Substance in Cell

Preliminary Report

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The Z-granules pointed out by the author[1] in the granulocyte series are characterised by the fact that they are demonstrated with thionin-sodium acetate as metachromatically stained yellow to brown.[2] Attention was now directed to the finding that the nucleolus of various cell types are stained with that dye in similar colors. The intracellular substance which shows the metachromasia will be called Z-substance.

Materials subjected were smears of blood and bone marrow fixed with methanol or susa solution, and paraffin sections of various tissues fixed with methanol-formol-glacial acetic acid (16:4:1) or Carnoy's solution.

Concerning the origin of Z-substance, the chromatin or histochemically DNA came to consideration among others because of the close quantitative and topographical relationships between them and also of the data reported in the previous papers.[3][4] In certain cell types such as basophile erythroblast, liver cell and nerve cell distinct figures were seen which suggested the emigration of Z-substance out of the nucleus into cytoplasm. As to the intracellular distribution of Z-substance, the granulocyte series, a part of promyelocytes excluded, represented rather a special case, in which the Z-substance was found intraplasmically as Z-granules, while it was absent in the nucleus.

Transformation of the Z-granules into Azur granules and basophile substance (RNA) was already referred to by the author.[1]

Details will be reported elsewhere.

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Studies on the Temperature of the Gastrointestinal Tract

Fourth Report

Influence of Ingestion of Water on the Intragastric Temperature

By

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(Received for publication, July 16, 1952)

Several reports[1)-5)] have been published concerning the influence of ingestion of hot and cold water upon the intragastric temperature of the normal stomach of men. However, no one has studied systematically the relation between temperature or volume of ingested water and change in the intragastric temperature. On the process of recovery of the latter, satisfactory reports are scanty. Paying attention to the above mentioned, I shall herein report on a systematical study of the said influence.

Experimental

Methods and Materials

100 experiments were carried out on 50 men and 17 women, who had no sign of gastrointestinal disorders. The intragastric temperature was measured in half-supine position by a thermometer[6)], which was introduced into the stomach at 60 cm. from the incisor of the upper jaw in the fasting state at least 4 hours after meals. It became almost constant about 30 minutes thereafter. Then water was introduced into the stomach through the Miller-Abbott's tube of the thermometer instead of ingesting per os to make the time for ingestion as constant as possible. For 30 minutes thereafter the intragastric temperature was continuously measured. Volume and temperature of the introduced water were as follows: 50, 150, 250, 350 and 450 cc. of 15–16°C water; and 150 cc. of 2–4, 24–25, 36–37 and 45–47°C water. As a control the intragastric temperature was measured only by introducing the thermometer into the stomach. Measuring of 10 individuals was carried out respectively in each of the above stated 9 combinations of temperature and volume of the introduced water and in the control. To compare with those results,

model experiments were performed using a rubber bag. After a thin oval rubber bag into which the same thermometer as before was introduced, was submerged in the water bath, the temperature of which is made equal to that surrounding the stomach, i.e., 37.1–37.6°C, water was introduced into the bag. Temperature and volume of the introduced water and the speed of its introduction were respectively the same as the corresponding human experiments. For 30 minutes thereafter the temperature in the bag was continuously measured keeping the temperature of the water bath constant and 5 measurings were repeated on each. The room temperature under which measurements were performed ranged between 18 and 24°C.

Results

The data of measurements of the intragastric temperature, which changed by introducing water into the stomach, are shown in Table I and those of the control in Table II. In the tables only the values of 0, 2, 6, 10, 18 and 30 minutes after introducing of water are given, and in Table I only the maximums, minimums, means and standard deviations of the changes of 10 individuals respectively in each of the 9 combinations of temperature and volume of the introduced water are shown. If in a semi-logarithmic table the time after introducing of water is given as abscissa and the mean of the change in the intragastric temperature in logarithmic scale (of course omitting minus signs) as ordinate, Fig. 1 is obtained in the group of 15–16°C water with the 5 kinds of volume and Fig. 2 in that of 150 cc. water with the 5 kinds of temperature. In case of the model experiments, Figures 3 and 4 are similarly obtained.

Discussion

At first the general views on the changes in the intragastric temperature after the introduction of water into the stomach are given. From the beginning of the introduction of water, the intragastric temperature abruptly dropped or rose according to the temperature of the introduced water, and immediately or at least 10–20 seconds after introducing, it reached the maximum change. It is important that the intragastric temperature, which does not reach that of the introduced water, is always higher than it in case of cold water and lower in case of hot water. As obviously in Table I, this gap of temperature becomes more remarkable proportionally to the difference between the temperature of the water and that in the stomach. Moreover, the quicker the introducing of water, the smaller the gap of temperature became—it is especially evident in the case of 2–4°C water. The cause, as it were, the preventing mechanism for overheating or -cool-

TABLE I

The Maximum (Max.), Minimum (Min.), Mean and Standard Deviation (St. d.) of Each of Temperature and Volume of the Introduced Water, Time for the Introduction, Intragastric Temperature (I.G.T.) before the Introduction and Changes in the I.G.T. 0, 2, 6, 10, 18 and 30 Minutes after the Introduction

	Temp. of water °C	Volume of water cc.	Time for introd. Sec.	I.G.T. before introd. °C	Changes in I.G.T. after introd. of water (minutes)					
					0 °C	2 °C	6 °C	10 °C	18 °C	30 °C
Max.	16.0	50	15	37.7	−14.2	− 7.7	− 2.8	−1.0	−0.3	−0.1
Min.	15.0	50	10	36.8	− 7.5	− 2.8	− 0.4	−0.2	0	0
Mean	15.5	50	12	37.24	−10.65	− 4.33	− 1.27	−0.49	−0.12	−0.03
St. d.					2.01	1.50	0.81	0.33	0.12	0.12
Max.	16.0	150	55	37.9	−17.0	−10.9	− 6.3	−3.4	−0.9	−0.3
Min.	15.0	150	35	37.1	− 8.2	− 3.7	− 2.0	−1.1	−0.2	0
Mean	15.6	150	45	37.38	−11.46	− 7.92	− 3.81	−1.92	−0.41	−0.09
St. d.					3.05	2.60	1.40	0.83	0.25	0.11
Max.	16.0	250	85	37.4	−18.5	−13.2	− 6.3	−3.2	−0.9	−0.3
Min.	15.0	250	65	36.7	−12.9	− 7.9	− 2.5	−0.8	−0.1	0
Mean	15.6	250	75	37.15	−16.88	−10.80	− 4.62	−1.95	−0.37	−0.04
St. d.					1.62	1.75	1.36	0.77	0.25	0.08
Max.	16.0	350	115	37.8	−19.6	−12.2	− 7.2	−4.2	−1.8	−0.3
Min.	15.0	350	100	37.2	−16.9	− 8.1	− 3.6	−1.2	−0.2	0
Mean	15.5	350	105	37.48	−18.50	−10.57	− 5.19	−2.81	−0.83	−0.17
St. d.					0.94	1.73	1.22	0.88	0.45	0.12
Max.	16.0	450	170	37.6	−21.2	−13.5	− 8.4	−6.3	−2.5	−0.8
Min.	15.0	450	135	37.2	−15.6	−10.6	− 4.5	−2.2	−0.4	−0.1
Mean	15.6	450	150	37.36	−17.66	−12.18	− 7.20	−4.43	−1.70	−0.40
St. d.					1.56	0.98	1.20	1.26	0.86	0.29
Max.	4.0	150	55	37.8	−28.1	−18.0	− 7.9	−5.2	−2.6	−0.9
Min.	2.0	150	35	36.9	− 9.8	− 7.0	− 2.0	−0.5	−0.2	0
Mean	3.0	150	45	37.40	−19.05	−11.21	− 5.59	−2.98	−1.26	−0.37
St. d.					7.53	3.10	2.00	1.64	0.87	0.31
Max.	15.0	150	55	37.8	−12.4	− 6.7	− 3.8	−2.3	−0.9	−0.2
Min.	24.5	150	40	36.7	− 8.2	− 3.3	− 1.2	−0.3	0	0
Mean	24.9	150	45	37.26	− 9.71	− 4.79	− 2.24	−1.02	−0.19	−0.02
St. d.					1.52	1.45	1.19	0.90	0.40	0.08
Max.	36.7	150	55	37.9	− 1.0	− 0.7	− 0.4	−0.3	−0.2	−0.2
Min.	36.0	150	35	37.0	− 0.2	− 0.1	0	0	0	0
Mean	36.5	150	45	37.50	− 0.59	− 0.27	− 0.07	−0.04	−0.02	−0.01
St. d.					0.34	0.19	0.13	0.13	0.12	0.11
Max.	47.0	150	55	37.9	8.4	5.4	2.8	1.8	0.8	0.3
Min.	45.0	150	35	37.2	4.7	1.8	0.7	0.2	0	0
Mean	46.0	150	45	37.50	6.62	3.23	1.21	0.59	0.20	0.04
St. d.					1.87	1.34	0.70	0.55	0.34	0.08

TABLE II

The Changes in the Intragastric Temperature of the Control

No.	Before °C	0 °C	2 °C	6 °C	10 °C	18 °C	30 °C
1	37.0	0	0	0	0	−0.1	0
2	37.5	0	0	−0.1	−0.1	−0.1	−0.1
9	37.3	0	0	0.1	0.1	0	0.1
16	37.2	0	0	0	0	0	0.1
17	37.5	0	0	0	0	0	0
44	37.7	0	0	0	0.1	0	0
45	37.0	0	0	0.1	0	0.1	0.1
52	37.8	0	0	0	0	−0.1	−0.1
53	37.3	0	0	0	0	−0.1	0
54	37.3	0	0	0	0	0.1	0.1
Sum	373.6	0	0	0.1	0.1	−0.2	0.2
Mean	37.36	0	0	0.01	0.01	−0.02	0.02

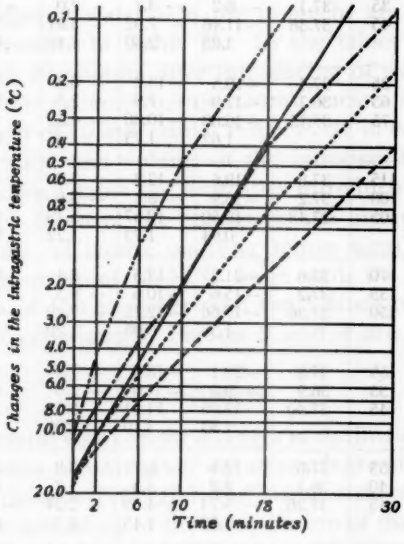

Fig. 1. The changes in the intragastric temperature (in logarithmic scale) after introducing of 15–16°C water with 50 (— · · — · · —), 150 (— · — · —), 250 (————), 350 (– – – – – –), and 450 cc. (— — — —). Minus sign in the temperature scale is omitted.

ing of the stomach, is difficult to explain but the heat which amounts to such an extent as in the case of 150 cc. of 2–4°C water is not lost through the Miller-Abbott's tube during the time for introducing, i.e., 45 seconds. Probably the stomach itself will play an important role during that time, for it has great expansion of the inner surface which is coated with

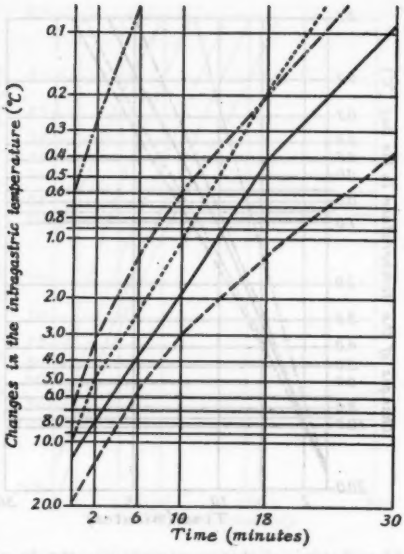

Fig. 2. The changes in the intragastric temperature (in logarithmic scale) after introducing of 150 cc. water in 2–4 (— — — —), 15–16 (——), 24–25 (- - - - - - - - -), 36–37 (— - - — - -), and 45–47°C (— - — -). Minus sign in the temperature scale is omitted.

the gastric mucus and abundant blood vessels. Soon after the above mentioned immediate drop or rise, the intragastric temperature began to recover at first rapidly then progressively slowly. Consequently in semi-logarithmic tables, Figures 1 and 2, the curves of recovery show almost straight lines. After mathematical procedures (the method of least squares, etc.), the following empirical formulas were obtained from the values of 0, 2, 4, 6, 10, 14, 18 and 22 minutes after introducing of water:

50 cc. of 15–16°C water.....$X^{50}_{15\text{-}16} = -\ 6.41\ e^{-0.225t}$,

150 cc. of 15–16°C water.....$X^{150}_{15\text{-}16} = -11.16\ e^{-0.177t}$,

250 cc. of 15–16°C water.....$X^{250}_{15\text{-}16} = -16.04\ e^{-0.205t}$,

350 cc. of 15–16°C water.....$X^{350}_{15\text{-}16} = -15.06\ e^{-0.162t}$,

450 cc. of 15–16°C water.....$X^{450}_{15\text{-}16} = -16.10\ e^{-0.127t}$,

150 cc. of 2–4 °C water.....$X^{150}_{2\text{-}4} = -14.63\ e^{-0.189t}$,

150 cc. of 24–25°C water.....$X^{150}_{24\text{-}25} = -\ 8.07\ e^{-0.208t}$,

150 cc. of 36–37°C water.....$X^{150}_{36\text{-}37} = -\ 0.34\ e^{-0.170t}$, and

150 cc. of 45–47°C water.....$X^{150}_{45\text{-}47} = \ \ 4.29\ e^{-0.170t}$; in which the change in the intragastric temperature t minutes after introducing of

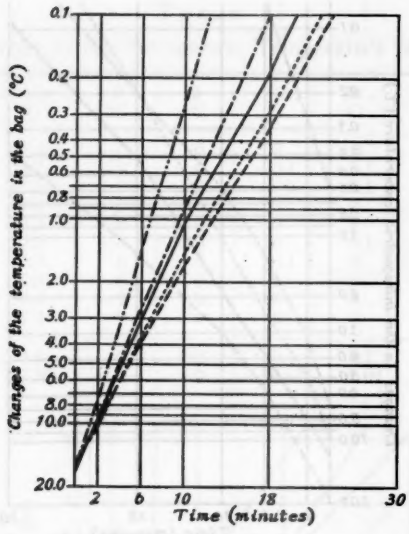

Fig. 3. The changes of the temperature in the bag in the model experiment which corresponds to Fig. 1.

water is X°C. As they are all exponential functions in which powers of " e " are the function of " $-t$ ", values of X theoretically approach endlessly towards zero with the rapse of time, thus the time for recovery must be infinite. However, as seen from Table II, in the control the intragastric temperature varies within ± 0.1°C without introducing of water. Therefore, the time for recovery is practically determined by the time when the intragastric temperature returns within about 0.1°C from the initial temperature. In fact, it was in a greater part about 30 minutes and among the values 30 minutes after introducing of water significant differences were mostly not seen contrary to the values immediately following the introduction.

Secondly the case in which the temperature of the introduced water is constant, i.e., 15–16°C, and with variable volume, is considered. In the experiment the time for introducing of water was made progressively long with approximately regular interval according to the volume. The drops in the intragastric temperature immediately after introducing were all smaller than the difference between the temperature of the water and that in the stomach, but were greater in the cases of 250, 350 and 450 cc. than 50 and 150 cc. (Fig. 1). As statistically significant differences are noticed between the formers and the latters but not among the formers and between the latters, the drops become different below and above 150

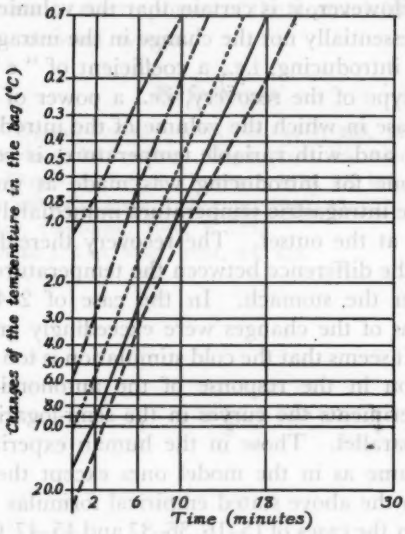

Fig. 4. The changes of the temperature in the bag in the model experiment which corresponds to Fig. 2.

and 250 cc. The standard deviations of the drops which are measures of individual variations in the experiment are greater in the cases of 50 and 150 cc. than the remaining three. However, in the model experiments (Fig. 3) the drops were almost equal in all cases from 50 to 450 cc., and the standard deviations were also almost equal and smaller. Next, the recovery after the above stated immediate drop became slower—the regression coefficient of its curve in the semi-logarithmic table became smaller—proportionally to the volume of water in the model experiments, and also in the human experiments with the exception of the case of 250 cc. Namely, in the human stomach, while among the regression coefficients in the cases of 50–450 cc. mutually significant differences are statistically noticed, the curve in the case of 250 cc. is so steep that it is crossed with that of 150 cc. as shown in Fig. 1. It is also interesting that in general the recovery in the human stomach is slower than in the model experiment. The chief differences between the human and model experiments are probably due to the reaction of the vessels of the stomach and its surroundings, the response of the gastric motility under the control of the autonomic nervous system, or the heat conductivity of the stomach to its surroundings, which may act at the same time. The specific behaviour in the case of 250 cc. seems to be related especially to the autonomic nervous system. Further remarks cannot be given at the present state

of experiments. However, it is certain that the volume of the introduced water determines essentially not the change in the intragastric temperature immediately after introducing, i.e., a coefficient of " e " of the empirical formula, but the type of the recovery, i.e., a power of " e ".

Thirdly the case in which the volume of the introduced water is constant, i.e., 150 cc. and with variable temperature, is considered. In the experiment the time for introducing was made as uniform as possible. The changes in the intragastric temperature immediately after introducing were as described at the outset. The recovery thereafter became slower proportionally to the difference between the temperature of the introduced water and that in the stomach. In the case of 2–4°C, however, the standard deviations of the changes were exceedingly great in comparison with the others. It seems that the cold stimulation is too intense to manifest individual variation in the response of the autonomic nervous system. In the model experiments the curves in the semi-logarithmic table (Fig. 4) are mutually parallel. Those in the human experiments can also be regarded as the same as in the model ones except their regression coefficients, because in the above stated empirical formulas the power of " e " is about -0.17 t in the cases of 15–16, 36–37 and 45–47°C and among them significant differences are not seen. Therefore, it is certain that the temperature of the introduced water essentially determines the change in the intragastric temperature immediately after the introduction of water—a coefficient of " e " of the empirical formula not a power of " e ".

Summary

I studied the changes in the intragastric temperature by introducing water into the stomach of healthy adults in comparison with those in the model experiments. Varying both temperature and volume of the water to be introduced, the following facts were confirmed.

1. The intragastric temperature abruptly drops or rises according to the temperature of the introduced water, but it does not reach the latter.

2. The recovery thereafter is expressed by an exponential function.

3. The time for recovery is practically decided by the time when the intragastric temperature returns within 0.1°C from the initial temperature.

4. The temperature of the introduced water essentially determines the change in the intragastric temperature immediately after the introduction of water and the volume of it governs the type of the recovery thereafter.

5. Those which deviate from the above cited relation between the intragastric temperature and temperature or volume of the introduced

water seem to be due to the responses of the stomach itself under the control of the autonomic nervous system.

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Histochemical Demonstration of the Acid Phosphatase in Erythrocytes

By

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Existence of the acid phosphatase in denucleated mammalian erythrocytes has been demonstrated, but only biochemically.[1][2] The enzyme, however, is demonstrable in rabbit erythrocytes almost constantly by the following histochemical technique.

Material: Fresh blood smear or bone marrow imprint.

Procedure: 1) Fix the specimens in 10% neutral formol saline or with formol vapor for 20 seconds at room temperature, 2) rinse gently in running tap water for 7 minutes, then in distilled water for 3 minutes, 3) incubate in Rabinovitch's substrate medium for acid phosphatase[3] for 8 hours at 37°C, 4) wash in running water for 10 minutes, 5) immerse in yellow ammonium sulfide diluted 1:40 for 2 minutes, 6) wash throughly in water and counterstain if desired, 7) dry in the air.

Results: All erythrocytes in the peripheral blood are stained homogeneously brown, while some of them contain a number of fine black granules, too. In the bone marrow, however, relatively weak diffuse staining and constant appearance of the granules are characteristic. It was noticed, in addition, that those granules are most probably the enzyme liberated by karyorrhexis or karyolysis in the normoblast stage.

Ordinary procedures,[4][5][6] especially fixation and incubation for longer duration, do not retain the enzyme. On the other hand, it was proved[7] that the positive results mentioned above are given by real enzymatic activity but neither by adsorption nor by non-specific precipitation. The enzyme in human erythrocytes does react, but only extremly faintly by the present technique.

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Studies on the Temperature of the Gastrointestinal Tract

Fifth Report

Response of the Intragastric Temperature to Various Drugs (Part I)

By

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(Received for publication, July 16, 1952)

In the previous reports[1)-3)], the responses of the intragastric temperature to bathing, local cooling and warming of the epigastrium, and ingestion of hot and cold water were studied. In this report responses of the said temperature to drugs acting on the autonomic nervous system and a gastric secretogogue are researched upon. Concerning this problem a report, in which histamine, acetylcholine, alcohol and typhoid vaccine were given to men, appeared by Thiessen and Snell[4)].

Experimental

Methods and Materials

Adrenaline, pilocarpine and atropine were selected as drugs acting on the autonomic nervous system and histamine as a gastric secretogogue. To observe their actions as purely and correctly as possible, a large dose was applied and attention was paid to avoid, as far as possible, variations may arise from weather, surroundings, nourishment and so on. First a routine table for experiments was made by arranging at random 24 combinations of a man and a drug which are obtained by injecting 1 mg. of histamine and adrenaline hydrochloride and atropine sulphate and 10 mg. of pilocarpine hydrochloride respectively to each of six healthy males whose ages ranged from 24 to 29 years, and then the experiments were performed according to it. The intragastric temperature was measured by a thermometer[5)], which was introduced into the stomach at 60 cm. from the incisor of the upper jaw in the fasting state at least 4 hours after meals. It became almost constant about 30 minutes thereafter. Then the drug was injected subcutaneously as above mentioned and for 60 minutes thereafter the intragastric temperature was continuously ob-

served. Simultaneously the axillary temperature was measured by a copper-constantan thermocouple and a potentiometer. Pulse rate and blood pressure were also determined at each 5 minutes to observe changes in the cardiovascular system, and gastric juice was aspirated at each 10 minutes through the Miller-Abbott's tube of the thermometer to observe response of the gastric secretion. The aspirated gastric juice was examined for the volume, free and total acidities by Toepfer's reagent and phenolphthalein solution and peptic power by Fuld-Levison's method[6]. Room temperature was ranged between 15 and 18°C.

TABLE I

The Changes in the Intragastric Temperature after the Injection of Histamine, Adrenaline, Pilocarpine and Atropine

Drug	No.	Before inject. °C	After injection (minutes) 5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	40 °C	50 °C	60 °C
Histamine	1	37.3	−0.15	−0.35	−0.55	−0.60	−0.55	−0.50	−0.30	−0.05	0.25
	2	37.4	−0.45	−0.50	−0.65	−0.65	−0.55	−0.45	−0.30	−0.15	−0.05
	3	36.8	−0.05	−0.40	−0.60	−0.65	−0.70	−0.65	−0.40	−0.15	−0.05
	4	37.8	−0.20	−0.35	−0.35	−0.35	−0.30	−0.25	−0.15	−0.05	0.05
	5	37.0	−0.50	−0.75	−0.75	−0.75	−0.65	−0.55	−0.40	−0.25	−0.20
	6	37.6	−0.20	−0.40	−0.50	−0.45	−0.45	−0.35	−0.25	−0.15	−0.05
	Sum	223.9	−0.92	−2.75	−3.40	−3.45	−3.20	−2.75	−1.80	−0.80	−0.05
	Mean	37.32	−0.15	−0.46	−0.57	−0.58	−0.53	−0.46	−0.30	−0.13	−0.01
Adrenaline	1	37.4	−0.10	−0.10	−0.10	−0.20	−0.25	−0.30	−0.35	−0.35	−0.35
	2	37.3	−0.10	−0.25	−0.25	−0.30	−0.35	−0.35	−0.30	−0.25	−0.25
	3	37.2	0	−0.15	−0.35	−0.45	−0.45	−0.50	−0.40	−0.35	−0.35
	4	37.4	0.05	0.05	0	−0.05	−0.10	−0.05	−0.10	−0.05	−0.05
	5	37.1	0	0	−0.05	−0.05	−0.10	−0.05	−0.10	−0.10	−0.05
	6	37.1	0	0	0	−0.05	−0.10	−0.15	−0.05	−0.10	−0.05
	Sum	223.5	−0.15	−0.45	−0.75	−1.10	−1.35	−1.40	−1.30	−1.20	−1.10
	Mean	37.25	−0.03	−0.08	−0.13	−0.18	−0.23	−0.23	−0.22	−0.20	−0.18
Pilocarpine	1	37.2	−0.05	−0.15	−0.25	−0.35	−0.45	−0.50	−0.55	−0.55	−0.45
	2	37.4	−0.05	−0.15	−0.15	−0.25	−0.35	−0.40	−0.50	−0.35	−0.25
	3	37.4	−0.05	−0.20	−0.35	−0.60	−0.70	−0.80	−0.85	−0.85	−0.80
	4	37.6	−0.05	−0.15	−0.50	−0.85	−0.85	−0.85	−0.70	−0.70	−0.65
	5	37.8	−0.05	−0.25	−0.45	−0.65	−0.65	−0.65	−0.60	−0.55	−0.45
	6	37.6	−0.05	−0.15	−0.25	−0.35	−0.45	−0.35	−0.35	−0.35	−0.35
	Sum	225.0	−0.30	−1.05	−1.95	−3.05	−3.45	−3.55	−3.55	−3.35	−2.95
	Mean	37.50	−0.05	−0.18	−0.33	−0.51	−0.58	−0.59	−0.59	−0.56	−0.49
Atropine	1	37.4	0	0	0.05	0	0.05	0.05	0.05	−0.10	−0.15
	2	37.4	0	−0.05	−0.05	−0.05	−0.05	−0.05	−0.10	−0.15	−0.10
	3	37.7	0	0	0.05	0.05	0.05	0.05	0.05	0	−0.05
	4	36.8	−0.05	0	0	0.05	0.05	0.05	−0.05	−0.10	−0.15
	5	37.4	−0.05	0	0.05	0.05	0.05	0.05	0	−0.05	−0.15
	6	37.5	0	0.05	0.10	0.10	0.10	0.05	0.05	−0.05	−0.05
	Sum	224.2	−0.10	0	0.20	0.30	0.25	0.20	0	−0.45	−0.65
	Mean	37.37	−0.02	0	0.03	0.05	0.04	0.03	0	−0.08	−0.11

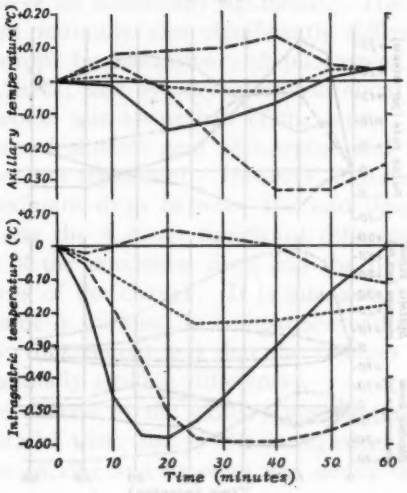

Fig. 1. The changes in the intragastric and axillary temperatures after the injection of histamine (———), adrenaline (- - - - - -), pilocarpine (— — — —) and atropine (— - — - —).

Results

The data for the changes in the intragastric temperature after the injection of the 4 drugs are given in Table I and Fig. 1. In Fig. 1 the means of the changes in the intragastric temperature are shown together with those in the axillary. The means of the changes in the systolic and diastolic blood pressures and pulse rate are illustrated in Fig. 2 and those of the changes in the gastric contents, i.e., those in the volume, free acidity and pepsin in Fig. 3.

Discussion

First the change in the intragastric temperature is discussed. Obviously in Fig. 1, following the injection of histamine the intragastric temperature began to drop abruptly and almost linearly reaching in the course of 15–20 minutes its maximum drop, −0.58°C on an average, and thereafter continued to rise also linearly recovering at 60 minutes after the injection. In cases of pilocarpine and adrenaline, it dropped almost linearly, reaching in the course of 30 minutes respectively the average maximum drops of −0.59 and −0.23°C and thereafter no significant change occurred. In case of atropine it at first rose then dropped on an average of −0.11°C in the course of 60 minutes. The above mentioned

served. Simultaneously the axillary temperature was measured by a copper-constantan thermocouple and a potentiometer. Pulse rate and blood pressure were also determined at each 5 minutes to observe changes in the cardiovascular system, and gastric juice was aspirated at each 10 minutes through the Miller-Abbott's tube of the thermometer to observe response of the gastric secretion. The aspirated gastric juice was examined for the volume, free and total acidities by Toepfer's reagent and phenolphthalein solution and peptic power by Fuld-Levison's method[6]. Room temperature was ranged between 15 and 18°C.

TABLE I

The Changes in the Intragastric Temperature after the Injection of Histamine, Adrenaline, Pilocarpine and Atropine

Drug	No.	Before inject. °C	After injection (minutes) 5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	40 °C	50 °C	60 °C
Histamine	1	37.3	−0.15	−0.35	−0.55	−0.60	−0.55	−0.50	−0.30	−0.05	0.25
	2	37.4	−0.45	−0.50	−0.65	−0.65	−0.55	−0.45	−0.30	−0.15	−0.05
	3	36.8	−0.05	−0.40	−0.60	−0.65	−0.70	−0.65	−0.40	−0.15	−0.05
	4	37.8	−0.20	−0.35	−0.35	−0.35	−0.30	−0.25	−0.15	−0.05	0.05
	5	37.0	−0.50	−0.75	−0.75	−0.75	−0.65	−0.55	−0.40	−0.25	−0.20
	6	37.6	−0.20	−0.40	−0.50	−0.45	−0.45	−0.35	−0.25	−0.15	−0.05
	Sum	223.9	−0.92	−2.75	−3.40	−3.45	−3.20	−2.75	−1.80	−0.80	−0.05
	Mean	37.32	−0.15	−0.46	−0.57	−0.58	−0.53	−0.46	−0.30	−0.13	−0.01
Adrenaline	1	37.4	−0.10	−0.10	−0.10	−0.20	−0.25	−0.30	−0.35	−0.35	−0.35
	2	37.3	−0.10	−0.25	−0.25	−0.30	−0.35	−0.35	−0.30	−0.25	−0.25
	3	37.2	0	−0.15	−0.35	−0.45	−0.45	−0.50	−0.40	−0.35	−0.35
	4	37.4	0.05	0.05	0	−0.05	−0.10	−0.05	−0.10	−0.05	−0.05
	5	37.1	0	0	−0.05	−0.05	−0.10	−0.05	−0.10	−0.10	−0.05
	6	37.1	0	0	0	−0.05	−0.10	−0.15	−0.05	−0.10	−0.05
	Sum	223.5	−0.15	−0.45	−0.75	−1.10	−1.35	−1.40	−1.30	−1.20	−1.10
	Mean	37.25	−0.03	−0.08	−0.13	−0.18	−0.23	−0.23	−0.22	−0.20	−0.18
Pilocarpine	1	37.2	−0.05	−0.15	−0.25	−0.35	−0.45	−0.50	−0.55	−0.55	−0.45
	2	37.4	−0.05	−0.15	−0.15	−0.25	−0.35	−0.40	−0.50	−0.35	−0.25
	3	37.4	−0.05	−0.20	−0.35	−0.60	−0.70	−0.80	−0.85	−0.85	−0.80
	4	37.6	−0.05	−0.15	−0.50	−0.85	−0.85	−0.85	−0.70	−0.70	−0.65
	5	37.8	−0.05	−0.25	−0.45	−0.65	−0.65	−0.65	−0.60	−0.55	−0.45
	6	37.6	−0.05	−0.15	−0.25	−0.35	−0.45	−0.35	−0.35	−0.35	−0.35
	Sum	225.0	−0.30	−1.05	−1.95	−3.05	−3.45	−3.55	−3.55	−3.35	−2.95
	Mean	37.50	−0.05	−0.18	−0.33	−0.51	−0.58	−0.59	−0.59	−0.56	−0.49
Atropine	1	37.4	0	0	0.05	0	0.05	0.05	0.05	−0.10	−0.15
	2	37.4	0	−0.05	−0.05	−0.05	−0.05	−0.05	−0.10	−0.15	−0.10
	3	37.7	0	0	0.05	0.05	0.05	0.05	0.05	0	−0.05
	4	36.8	−0.05	0	0	0.05	0.05	0.05	−0.05	−0.10	−0.15
	5	37.4	−0.05	0	0.05	0.05	0.05	0.05	0	−0.05	−0.15
	6	37.5	0	0.05	0.10	0.10	0.10	0.05	0.05	−0.05	−0.05
	Sum	224.2	−0.10	0	0.20	0.30	0.25	0.20	0	−0.45	−0.65
	Mean	37.37	−0.02	0	0.03	0.05	0.04	0.03	0	−0.08	−0.11

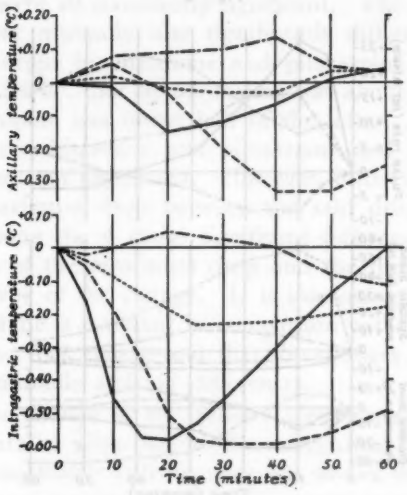

Fig. 1. The changes in the intragastric and axillary temperatures after the injection of histamine (———), adrenaline (- - - - - -), pilocarpine (— — — —) and atropine (— - — - —).

Results

The data for the changes in the intragastric temperature after the injection of the 4 drugs are given in Table I and Fig. 1. In Fig. 1 the means of the changes in the intragastric temperature are shown together with those in the axillary. The means of the changes in the systolic and diastolic blood pressures and pulse rate are illustrated in Fig. 2 and those of the changes in the gastric contents, i.e., those in the volume, free acidity and pepsin in Fig. 3.

Discussion

First the change in the intragastric temperature is discussed. Obviously in Fig. 1, following the injection of histamine the intragastric temperature began to drop abruptly and almost linearly reaching in the course of 15–20 minutes its maximum drop, −0.58°C on an average, and thereafter continued to rise also linearly recovering at 60 minutes after the injection. In cases of pilocarpine and adrenaline, it dropped almost linearly, reaching in the course of 30 minutes respectively the average maximum drops of −0.59 and −0.23°C and thereafter no significant change occurred. In case of atropine it at first rose then dropped on an average of −0.11°C in the course of 60 minutes. The above mentioned

Fig. 2

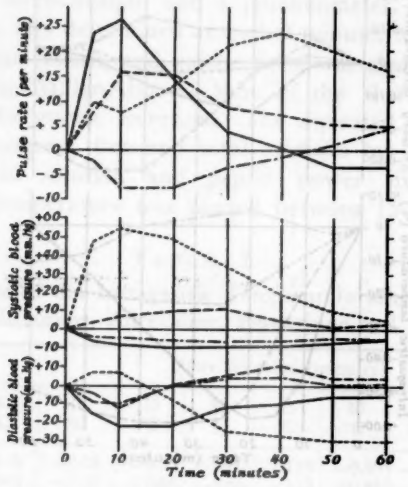

Fig. 3

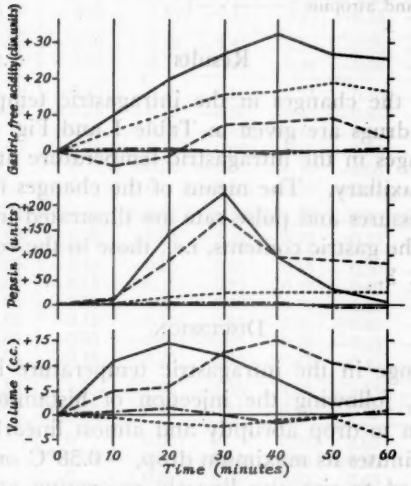

Fig. 2. The changes in the pulse rate and systolic and diastolic blood pressures after the injection of histamine (———), adrenaline (- - - - - -), pilocarpine (— — — —) and atropine (— - — - — - —).

Fig. 3. The changes in the free acidity, pepsin and volume of the aspirated gastric juice after the injection of histamine (———), adrenaline (- - - - - -), pilocarpine (— — — —) and atropine (— · — · — · —).

drops and rises were all statistically significant. The types of the change (time series) were mutually also significantly different by the 4 drugs. The maximum drops by histamine and pilocarpine were almost equal (−0.58 and −0.59°C), that by adrenaline was about half of the formers, and that by atropine was about half of the latter. In these drops the differences between histamine and pilocarpine and between adrenaline and atropine were not significant. However, differences of the time for reaching the maximum drop between the said drugs were significant. Accordingly, among the 4 drugs significant differences were noticed in the combination of the maximum drop and the time for reaching it as well as in the type of the change. It is interesting that in general the change by histamine is transient in comparison with the drugs acting on the autonomic nervous system and that the changes by pilocarpine and atropine show mutually striking differences.

Secondly the change in the axillary temperature is considered. It was generally parallel with that in the intragastric (Fig. 1). However, its magnitude was smaller and its onset was slower than the latter; and the ratio of the magnitudes of both temperatures was different from one another by the 4 drugs. The combination of the maximum change and the time for reaching it was, similarly as in the case of the intragastric, significantly different by the 4 drugs. As the axillary temperature is greater in individual variation and inferior in the accuracy of measurement than the intragastric, it is difficult to conclude from the change of the former comparing with the latter.

Thirdly the change in the cardiovascular system is considered. The pulse rate was significantly increased by histamine, pilocarpine and adrenaline, but by atropine it decreased as described in the monograph by Krantz and Carr[7] (Fig. 2). Onset of the change in the pulse rate precedes that in the intragastric temperature and among the 4 drugs significant differences are seen in the combination of the maximum change and the time for reaching it as well. The changes in the systolic and diastolic blood pressures were not directly related with those in the intragastric temperature as seen in Fig. 2. It is worthy of note that the change in the pulse rate was in a good contrast with that in the intragastric temperature. Namely, according to the increase or decrease of the pulse rate the intragastric temperature inversely drops or rises and between the magnitudes of both changes a good proportion is noticed. After the injection of histamine, the face and skin became flushed and the vessels of the skin dilated. Therefore, it is postulated that because the blood which is cooled peripherally at the body surface and the lungs returns to the heart and flows into the vessels of the stomach and its surroundings, the intragastric temperature is lowered; and that as the action of histamine

is relatively transient, the drop of the intragastric temperature will be also temporary. In case of the drugs acting on the autonomic nervous system changes in the cardiovascular system are more complicated, but it will be certain that the change in the blood flow is one of the most important factors. According to this assumption the change in the axillary temperature is also easily explained. Importance of the blood circulation in the change in the intragastric temperature was already stressed in cases of bathing and of cooling or warming of the epigastrium in the second and third reports[1)2)]. From the present experiments the importance that the intragastric temperature is in intimate relation with the blood flow, is also evident.

Fourthly the change in the gastric juice is discussed. Among the changes shown in Fig. 3, only those in the free acidity by histamine and in the pepsin by histamine, pilocarpine and adrenaline are significant, owing to the lack of a reliable method for determination of the change in the gastric juice and the great individual variation. However, tendencies of the changes are noticed. Namely, the volume of the secretion and pepsin were increased by histamine and pilocarpine and the free acidity by histamine. It is interesting that by the drugs which intensely stimulate the gastric secretion, i.e., histamine and pilocarpine, the intragastric temperature is also remarkably lowered. Regarding to the relation between the intragastric temperature and the gastric secretion, many possibilities, for example, accelerated gastric blood flow, have been considered, but under the present state it is difficult without further studies to explain clearly the relation.

Summary

Response of the intragastric temperature of healthy men to subcutaneous injection of histamine, adrenaline, pilocarpine and atropine was studied simultaneously with those of the axillary temperature, pulse rate, blood pressure and gastric juice; and the following facts were confirmed.

1. The intragastric temperature drops by the four drugs.
2. The drop by histamine is transient contrary to those by adrenaline and pilocarpine and the drop by atropine is preceded by a slight rise.
3. The maximum drop is large by histamine and pilocarpine and small by adrenaline and atropine.
4. Onset of the drop is more rapid by histamine than by pilocarpine and adrenaline and is slowest by atropine.
5. Combination of the maximum drop and the time for reaching it, is different by the four drugs.
6. Change in the axillary temperature is generally parallel with

that in the intragastric.

7. Change in the pulse rate is in intimate relation with that in the intragastric temperature. Blood flow is one of the most important factors in the change in the intragastric temperature.

8. By histamine and pilocarpine which strongly stimulate the gastric secretion, the intragastric temperature is intensely lowered.

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On the Chemical Properties of the Z-Granules

III. Test by Pyronin-Methylgreen Staining

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(Received for publication, February 3, 1953)

Imprints of rabbit bone marrow were fixed with methanol and then stained with aqueous solution of methylgreen (1%) or/and pyronin (1%) purified with buthanol, with results in Table I.

TABLE I

Dye and submerging time	Color of stains		
	DNA	Z-granules[1-4]	RNA
Methylgreen only (5 min.)	+ greenish blue	− unstained	± greenish blue
Pyronin only (5 min.)	+ red	### yellow to reddish yellow*	### red
Pyronin, then methylgreen (5 min.) : (1 min.)	+ greenish blue	### reddish yellow*	### red
" " " (5 min.) : (2.5 min.)	+ greenish blue	## red	## red
" " " (5 min.) : (over 15 min.)	+ greenish blue	+ reddish violet (mixed color)	## reddish violet (mixed color)

(time): duration of staining; ### most intensive, ## intensive, + moderately intensive, ± rarely and faintly stained. * If a lower concentrated pyronin solution is used, much longer time was requisite to demonstrate the metachromasia.

It has been already informed by Yamasaki[3)4)] that both of Z-granules and hydrolysed DNA stain similar with thionin metachromatically; the finding here indicates that pyronin gives the same stain.

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Studies on the Temperature of the Gastrointestinal Tract

Sixth Report

Response of the Intragastric Temperature to Various Drugs (Part II)

By

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(Received for publication, July 16, 1952)

In the previous report[1], the response of the intragastric temperature of healthy men to adrenaline, pilocarpine, atropine and histamine was studied. As a sequel to it, using other drugs acting on the autonomic nervous system, especially autonomic blocking drugs, and also repeatedly histamine, a further study concerning the said response is presented herein.

Experimental

Methods and Materials

The intragastric temperature was measured simultaneously with the pulse rate, blood pressure and gastric juice after the same procedures as previously reported[1]. Four drugs were injected in the following six ways: subcutaneous injection of 0.8 and 0.4 mg. per kg. of benzylimidazoline hydrochloride, 0.01–0.02 mg. per kg. of prostigmine methylsulfate and 0.0175 mg. per kg. of histamine hydrochloride; and intravenous injection of 0.6–0.7 mg. per kg. of benzylimidazoline hydrochloride and 6–8 mg. per kg. of tetraethylammonium bromide (TEAB). For each of the mentioned six ways, 10 individuals each, totaling 60 adults including 42 men and 18 women of 19 to 48 years of age, who had no previous sign of gastrointestinal disorders, were tested. The room temperature under which the experiments were performed ranged between 13 and 23°C.

Results

The data for the changes in the intragastric temperature after the injection of the four drugs in the six ways are given in Table I and Fig. 1. In Table I the upper and lower limits and means of the changes at

TABLE I

The Upper (U.L.) and Lower Limits (L.L.) and Means of the Changes in the Intragastric Temperature at Each Time after the Injection of Benzylimidazoline, Tetraethylammonium Bromide (TEAB), Prostigmine and Histamine

Drug and mode of injection		Dose mg./kg.	Before inject. °C	After injection (minutes)								
				5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	40 °C	50 °C	60 °C
Benzyl-imidazoline subcutan.	U.L.	0.4	37.9	0	0	0	−0.05	−0.05	−0.05	−0.10	−0.10	−0.10
	L.L.	0.4	36.9	−0.15	−0.15	−0.20	−0.25	−0.30	−0.35	−0.40	−0.40	−0.45
	Mean	0.4	37.37	−0.02	−0.06	−0.10	−0.12	−0.14	−0.15	−0.19	−0.22	−0.24
Benzyl-imidazoline subcutan.	U.L.	0.8	37.7	0	−0.05	0	−0.05	−0.05	−0.15	−0.25	−0.20	−0.25
	L.L.	0.8	37.0	−0.20	−0.35	−0.50	−0.65	−0.80	−0.80	−0.80	−0.70	−0.65
	Mean	0.8	37.38	−0.07	−0.18	−0.22	−0.32	−0.36	−0.40	−0.43	−0.46	−0.44
Benzyl-imidazoline intraven.	U.L.	0.7	37.9	−0.10	−0.15	−0.15	−0.20	−0.15	−0.15	0	0	0
	L.L.	0.6	36.8	−0.30	−0.50	−0.50	−0.55	−0.45	−0.40	−0.45	−0.40	−0.45
	Mean	0.627	37.47	−0.19	−0.28	−0.30	−0.32	−0.28	−0.26	−0.22	−0.20	−0.16
TEAB intraven.	U.L.	8.0	37.9	0	−0.10	−0.10	−0.15	−0.15	−0.10	−0.05	0.05	0.10
	L.L.	6.0	36.8	−0.30	−0.40	−0.50	−0.40	−0.45	−0.40	−0.35	−0.35	−0.35
	Mean	7.06	37.23	−0.12	−0.23	−0.28	−0.28	−0.28	−0.26	−0.23	−0.17	−0.12
Prostig-mine subcutan.	U.L.	0.02	37.8	0	0.05	0.05	0.05	0.10	0.15	0.25	0.25	0.25
	L.L.	0.01	37.0	−0.10	−0.05	−0.05	−0.05	−0.05	0	0.05	−0.05	0
	Mean	0.016	37.51	−0.02	0.01	0	0	0.01	0.04	0.14	0.08	0.07
Histamine subcutan.	U.L.	0.0175	37.8	−0.15	−0.35	−0.35	−0.35	−0.30	−0.25	−0.15	0	0.25
	L.L.	0.0175	37.0	−0.50	−0.75	−0.75	−0.75	−0.75	−0.70	−0.60	−0.45	−0.35
	Mean	0.0175	37.45	−0.28	−0.46	−0.56	−0.56	−0.52	−0.44	−0.30	−0.18	−0.07

each time after the injection are shown and in Fig. 1 the means are plotted. The means of the changes in the pulse rate and systolic and diastolic blood pressures are illustrated in Fig. 2 and those in the gastric free acidity in Fig. 4. Concerning Fig. 3, refer to the following discussion.

Discussion

First the change in the intragastric temperature is considered. As shown in Fig. 1, by subcutaneous injection of benzylimidazoline the intragastric temperature continued to fall gradually and showed no perceptible signs of recovery within 60 minutes after the injection. Its maximum drop in the case of 0.4 mg. per kg. was −0.24°C on an average and in the case of 0.8 mg. −0.46°C, about twice as much as the former. The curve of its fall was significantly steeper in the latter than in the former, though both showed a similar tendency. By the intravenous injection the fall was rapid in the onset contrary to the subcutaneous one. Namely, the intragastric temperature reached its maximum drop of −0.32°C in

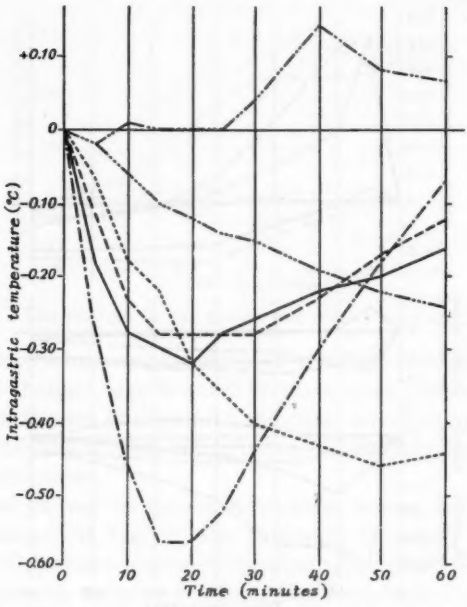

Fig. 1. The changes in the intragastric temperature after the subcutaneous injection of benzylimidazoline (0.4 mg. per kg.: — · · · —, 0.8 mg.: · · · · · · · ·), prostigmine (— · · — · · —) and histamine (— · · — · · —); and the intravenous injection of benzylimidazoline (————————) and tetraethylammonium bromide (— — — — — —).

the course of the first 20 minutes, and thereafter it began to recover slowly. Therefore, the drop by benzylimidazoline is generally proportional to the injected dosis regardless of the mode of injection but its curve is obviously different by the mode. In the case of TEAB approximately the same curve of the change as the intravenous injection of benzylimidazoline was observed. This is interesting when compared with the fact that, though both TEAB and benzylimidazoline are autonomic blocking drugs, their actions differ from each other. The drop by histamine was greatest, reaching its maximum, —0.56°C, in the course of the first 15 minutes, and both the onset and the recovery were most rapid in spite of the subcutaneous injection. In the case of prostigmine, contrary to the above mentioned, the intragastric temperature showed no significant change for the first 20–25 minutes and thereafter it began to rise. It reached the maximum of 0.14°C, at 40 minutes after the injection and then recovered slowly. In our experiences there are only a few drugs, by which the intragastric temperature rises, and the rise by prostigmine is interesting when

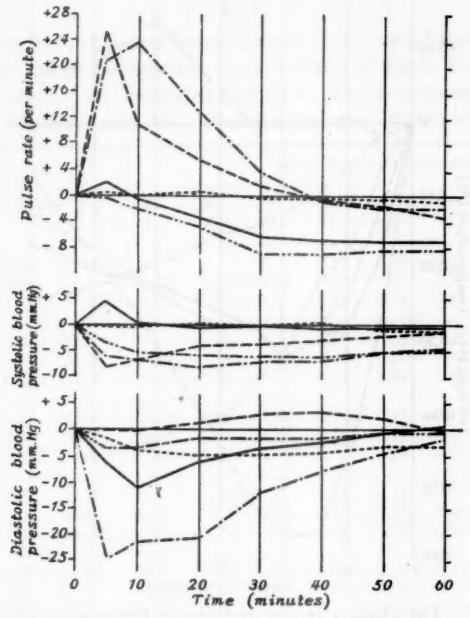

Fig. 2. The changes in the pulse rate, systolic and diastolic blood pressures as noted in Fig. 1.

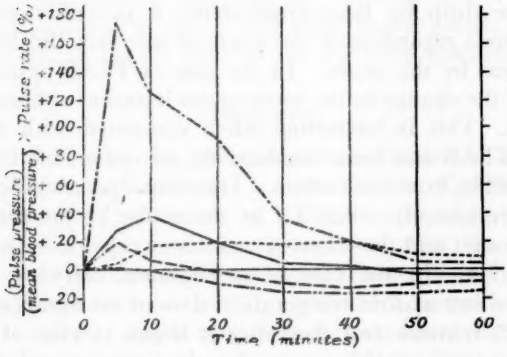

Fig. 3. The changes in the $\frac{\text{(pulse pressure)}}{\text{(mean blood pressure)}} \times \text{(pulse rate)}$ as noted in Fig. 1.

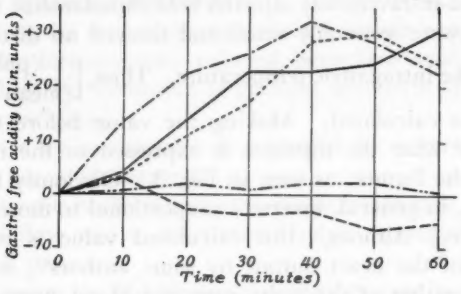

Fig. 4. The changes in the gastric free acidity as noted in Fig. 1.

compared with the initial rise by atropine reported previously[1]. Among the above stated changes, significant differences were noticed in the combination of the maximum change and the time for reaching it as well as in the type of the change, except between TEAB and intravenously injected benzylimidazoline.

Secondly the change in the cardiovascular system is discussed. The pulse rate, as shown in Fig. 2, was strikingly increased by TEAB and histamine at 5–10 minutes after the injection, i.e., 24–25 per minute, and thereafter it began to recover, reaching the level before the injection in the course of 30 minutes. The increase by TEAB was more rapid in reaching the maximum than histamine, probably due to intravenous application. Contrary to the above changes, by prostigmine and intravenously injected benzylimidazoline the pulse rate gradually decreased with or without an initial slight increase and reached the maximum decrease, 7–9 per minute, in the course of 30 minutes, remaining thereafter on the same level up to 60 minutes after the injection. By the subcutaneous injection of benzylimidazoline, however, the pulse rate showed no significant change throughout 60 minutes. The inverse proportion between the change in the intragastric temperature and that in the pulse rate emphasized in the previous report[1], is generally not noticed in these drugs. Namely, by prostigmine and intravenously injected benzylimidazoline, by which the pulse rate showed almost the same change, the intragastric temperature changed with mutually different directions—one rised and the other dropped; and by TEAB and intravenously injected benzylimidazoline the changes in the pulse rate differed from each other, while those in the intragastric temperature were approximately the same. Furthermore, by subcutaneously injected benzylimidazoline the pulse rate did not change significantly, while the intragastric temperature gradually fell. Next, among the changes in the blood pressure, only those in the systolic by histamine and TEAB and those in the diastolic

by histamine and intravenously injected benzylimidazoline were significant. However, they were generally small and showed no direct relation with the changes in the intragastric temperature. Then, $\left[\frac{\text{(pulse pressure)}}{\text{(mean blood pressure)}}\right] \times$(pulse rate) is calculated. Making the value before the injection as 100, the change after the injection is expressed as the relative increase or decrease to the former, as seen in Fig. 3. Obviously from the figure, the changes are, in general, inversely proportional to those in the intragastric temperature. Although this calculated value is estimated as an approximation of the heart output by some authors[2], it is certain that this is an aggregation of the pulse rate and blood pressure. Therefore, the change in the intragastric temperature is determined by the pulse rate and blood pressure. From the present experiments the importance that the intragastric temperature is in intimate relation with the blood flow, is also evident, as previously reported[1].

Thirdly the change in the gastric juice is discussed. The gastric free acidity rose significantly in the cases except TEAB and prostigmine. However, by TEAB it tended to decrease. It is interesting that among the rises by histamine and benzylimidazoline (both by subcutaneous and intravenous injection), significant differences were not seen contrary to the falls in the intragastric temperature; and that entirely different changes in the free acidity were observed between TEAB and intravenously injected benzylimidazoline, by which the intragastric temperature changed tracing almost the same curve. Furthermore, between the curves of the changes in the intragastric temperature and in the free acidity, no significant proportion was seen. Therefore, it is concluded that the change in the gastric free acidity is not related with that in the intragastric temperature. Additionally, as a gastric secretogogue, benzylimidazoline which is smaller in the changes in the cardiovascular system than histamine, can be used instead of histamine.

Summary

Response of the intragastric temperature of healthy men to subcutaneous injection of benzylimidazoline, prostigmine and histamine and to intravenous injection of benzylimidazoline and tetraethylammonium bromide was studied, simultaneously with those of the pulse rate, blood pressure and gastric juice; and the following facts were confirmed.

1. The intragastric temperature drops by benzylimidazoline, tetraethylammonium bromide and histamine, and rises by prostigmine.

2. The drop by benzylimidazoline is generally proportional to the injected dosis but the type is different by the mode of injection—by the subcutaneous injection the drop gradually increases and by the intrave-

nous it rapidly appears and recovers.

3. The drop by tetraethylammonium bromide is approximately the same as that by intravenously injected benzylimidazoline.

4. The drop by histamine is greatest and both the onset and the recovery are most rapid.

5. The rise by prostigmine is preceded by a certain period when no significant change is observed.

6. The change in the intragastric temperature is in intimate relation with the blood flow and is determined by an aggregation of the pulse rate and blood pressure.

7. The change in the intragastric temperature is not related with that in the gastric free acidity.

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The Relationship between Blue Nevus and Vitiligo

By

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Case report. 42 years old female. About half a year ago, a small leucoderma appeared on the nape which gradually enlarged and extended to the scapular region. Upon inspection, an irregular shaped leucodermatous patch was noticed on the right upper back. Most of the lesion localized on the right while some extended to the left beyond the spine. No increase of pigment on the border. At the inner margin of the right scapula in the vitiliginous area, a rice grain sized pigment spot of light blue hue was noticed. Clinical diagnosis: Vitiligo with blue nevus?

Histological findings in the superficial layer were that of usual vitiligo. In the corium, several elongated pigment cells were noticed in the middle reticular layer. These cells contained coarse melanin granules and were situated between connective tissue fibers. They corresponded with blue nevus cells or Mongolian spot cells in their morphology and arrangement and can be readily distinguished from chromatophores. In dopa specimens, no melanoblasts were seen in the epidermis. In the corium, some of the blue nevus cells were dopa-positive.

Discussion. The existence of blue nevus alongside of vitiligo reminds one of leucoderma centrifugum aquisitum Sutton. In our case, however, it is proper to assume that vitiligo had invaded the pre-existent blue nevus. Quite interesting is the fact that blue nevus cells still showed positive dopa reaction in contrast to the negative one in the epidermis. This suggests that there exists certain, at least, chronological difference in the reaction of ectodermal and mesodermal melanoblasts toward unknown noxaecausing depigmentation. Prof. Itô* proposed that blue nevus cells are of mesodermal origin derived from perineural mesenchymal tissue and he surmised that melanogenesis is interpreted as a neurohormonal reaction. Our observation indicates possible difference in the regulative mechanism of melanogenesis in the ectodermal and mesodermal melanoblasts and is in favor of his theory. However, such case is rare and we can find no similar report in the literature. So we wait for further confirmation by investigators in this field.

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Histological Study on Distribution of Aortic Nerve in Human Embryo

By

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(Received for publication, July 21, 1952)

In 1866, Cyon and Ludwig[1] observed emergence of reduced blood pressure when the nerve bundle running toward arcus aortae in rabbit is mechanically incited. They named this nerve n. depressor, under the impression that it acted centrifugally. However, Hering,[2] in his famous physioligical experiment on the subject in 1924, proved that this phenomenon of reduced blood pressure is caused reflexively, that is, this nerve is not centrifugal, but centripetal in nature. Moreover, he succeeded in ascertaining that such depression of blood pressure is caused also by a nerve of similar nature ending in sinus caroticus, which he christened the sinus nerve. At the same time, he changed the name of n. depressor to aortic nerve.

This physiological experiment was confirmed by many succeeding physiologists, and then many histologists also, including Tello,[3] de Castro,[4] Tschernjachiwsky,[5] Sunder-Plassmann,[6] Muratori,[7] Stöhr,[8] Nonidez,[9] Palme[10] and Takino[11] studied the terminal formations of both the above nerves with their own silver methods and reported the results.

In 1930, de Castro[4] and Sunder-Plassmann[6] independently succeeded in observing the existence of special sensory terminations in sinus caroticus of men and animals, peculiar in that they differed considerably in conformation from sensory terminations found in other artery walls. This fact was later on endorsed by their successors, including Stöhr[8] and Muratori.[7]

The existence of sensory terminations in arcus aortae was afirmed by Tello[3] in 1924 and Tschernjachiwsky[5] in 1929–30 chiefly in mouse fetus, by the latter, only expounded in one figure. Palme[10] (1934), Muratori[7] (1935) and Nonidez[9] (1935) made successive studies on the subject from histological standpoint. But all these studies have not given observation in perfect agreement with the results of Sunder-Plassmann's studies on sinus caroticus, for the former used animals, new-born infants

or embryos as material, whereas Sunder-Plassmann used human adults in his study. This difference in material led to the failure of the former to observe anything but much simpler terminations in arcus aortae than in sinus caroticus.

Of the both nerves connected with the reflex depression of blood pressure, the terminations of sinus nerve in adults were described by Sunder-Plassmann and de Castro, while the terminal formations of aortic nerve in adults, which was known for a longer time, were reserved to Seto[12] for full description in 1937. He succeeded not only in proving the existence of terminations in perfect correspondence with Type I and Type II of the peculiar terminations described in sinus caroticus by Sunder-Plassmann, in the lower wall of arcus aortae in a large number, but also a small number of such peculiar terminations in the human heart atria. Thus it was assured that the sinus nerve and the aortic nerve are sensory nerves in perfect conformity, both physiologically and histologically.

In 1933, Sunder-Plassmann[6] observed a small quantity of sensory terminations corresponding to Type I above in lamina muscularis mucosae of small pulmonary bronchi in man. This led Seto to conclude that these peculiar terminations are also common to the other organs closely related with the circulation of blood, beside the above mentioned major artery walls. In endorsement of this supposition, Seto's desciples discovered the conspicuous presence of this Type I and Type II terminations in the mucous membrane of larynx (Sasaki[13] and Matsumoto)[14] and in the muscular layer of trachea (Sasaki)[13].

There are very few of blood vessels, in particular, of arteries provided with sensory terminations, these being limited to coronal arteries, cerebral arteries, small arteries in pia mater and femoral arteries. The terminations in the two former are represented as simple branched terminations and in the two latter as glomerular terminations. But the terminations of the sensory nerves acting reflexively in reducing blood pressure are quite different from these in conformation and were classified by Seto into two types. The first type is represented by complex branched terminations provided with peculiar neurofibrillar terminal plates, while the Type II is represented by complex ramified terminations mostly accompanied by small arteries.

As described above, the formation of sensory terminations connected with the reflexive reduction of blood pressure in adults has been very clearly elucidated, but little was done in studying the formation embryologically and systematically, with human embryo as material. This fact comes presumably from the difficulty of silver-staining for the nerves. I undertook to make study on 4th, 6th and 8th month embryos, to throw some light on this matter. I took their hearts together with large blood

vessels connected thereto and fixed them for a long time in 10% neutral formol. These I cut into 40 μ horizontal frozen sections and stained with Seto's silver method. The series of these beautifully stained preparations I scrutinized minutely under a microscope and arrived at the observations as outlined hereunder.

Individual Views

I will preface my observations on the distribution as well as terminal formation of aortic nerve in human embryo with some citations of previous studies made on human and animal embryos. Firstly, Palme (1934)[10] depicted only one rather dubious branched ending in the media of arcus aortae of a 33 cm human embryo in one of his illustrations, and remarked that this might be a terminal formation of aortic nerve.

In 1935, Muratori[7] discovered the terminations of aortic nerve in the media of arcus aortae of a 6th month human embryo and described them also with one illustration, but from his observations in rabbits, mice and cats, all new-born, concluded that such terminations could be classified into two types. Tello (1924)[3] and Tschernjachiwsky (1929–30)[5] also used small animals as material, and it may be said that some light has been thrown on the problem, insofar as it concerns with animal fetus. On the other hand, the study of the problem regarding human embryo seems to have been left in the cold.

In human adults, the terminations of aortic nerve are chiefly formed in the lower edge of arcus aortae and to a smaller extent in the atria cordis. They are found in arcus aortae only in the connective tissue terminal area of the externa bulging into the media, and not in the media further inside (Seto).[12] According to Sunder-Plassmann[6] and Ohtomo,[15] this is also the case by carotid sinus. But in my study of human embryos I found much deviation from such observations on adults, both in the distribution of aortic nerve and in the terminal formation of its nerve fibres.

In examining my silver-impregnated preparations of the emergent parts of the large blood vessels going out from the hearts of human embryos, I found, in the 4th month embryo already, many well-developed sensory fibres spreading out all over the wall of arcus aortae from the lower edge thereof, running further down between aorta and a. pulmonalis, or distributed along the pulmonary arteries on both sides, furthermore penetrating the media and sometimes even the intima, to terminate in extremely peculiar formed branched terminations over a large area.

These terminations originate in the centripetal fibres of aortic nerve coming from n. vagus. The small nerve bundles found in the wall of the artery are not composed solely of thick medullated sensory nerve

fibres, containing many thin vegetative fibres. This fact was observed with extreme clarity in the connective tissue externa on the outside of the media at the lower edge of arcus aortae of 8th month embryo, as shown in Fig. 1.

To enter into further details regarding the distributing area of the aortic nerve in human embryo, it comprises not only the lower edge of the aorta but also its entire wall, the ascending aorta, especially its dorsal inner wall, the emergent parts of truncus brachiocephalicus, a. carotis comm. sinistra and aa. pulmonales. However, this broad development of the nerve seem to be more marked at the earlier stage of embryonic life, showing gradual degeneration in the later stage, especially in regard to the pulmonary artery. Thus, in 4th month embryo, the terminations in the pulmonary artery show a very marked development especially in the media, whereas in 8th month embryo, only a very small quantity of terminations is found there, almost none of them penetrating into the media. In the aorta and emergent parts of its major branches, sensory terminations are better developed than in a. pulmonalis, but here also the number of terminations penetrating into the media is much smaller in 8th month embryo than in 4th month one. It seems that the distributing area of aortic nerve undergoes a slow narrowing down, until it becomes limited to the lower edge of arcus aortae in adults.

I will begin my description of the terminal formations of aortic nerve found in arcus aortae and aorta ascendens. What is remarkable about these formations is that they are all formed in the smooth muscle layer of the media, very few of them ending in the externa, as in adults, and that the terminal branches spread out along the smooth muscle fibres arranged circularly. Consequently, only cut parts of nerve fibres are met with in preparations of longitudinal sections of aorta, but in its cross-sections terminations are shown with all clarity.

The terminations in human adults are classifiable, as stated above, into Type I provided with a number of neurofibrillar terminal plates and Type II without such plates but always accompanying small arteries. But as these are found only in the externa, I presumed that the terminations found in the aorta of embryos need not be in exact histological conformability with those in adults. I was confirmed in my expectation in my study, as under minute scrutiny, the terminations in embryos showed a widely different conformation from those of the adults.

Some of the thick sensory fibres, upon penetrating into the media, undergo repeated ramifications, to pass into arborized terminations as seen in Fig. 2 and Fig. 3, while others pass into undeterminate terminations as shown in Fig. 4. The former are more conspicuous in the later stage of the embryonic life, while the latter are more in evidence

Fig. 1

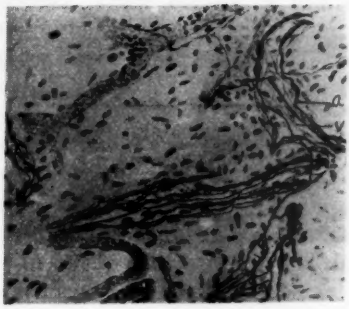

Fig. 2

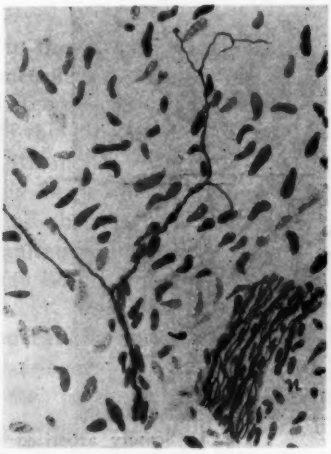

Fig. 1. Small bundles of aortic nerve penetrating into the externa of the lower edge of arcus aortae in 8th month human embryo. a thick sensory fibres: v thin vegetative fibres. Seto's silver impregnation. X 280, reduced to 2/5.

Fig. 2. Simple arborized termination originated in aortic nerve formed in media of lower edge of arcus aortae in 8th month human embryo. n a small bundle of aortic nerve composed of thick sensory and fine vegetative fibres, found in the externa covering the media. Same staining. X450, reduced to 1/2.

in the earlier stage. Both are utterly different from terminal formations seen in adults and are incomparably simpler. But in quantity, these terminations surpass by far those in adults.

The nerve fibres, especially the terminal branches, are very typical in that they show very conspicuous change in thickness. Of particular interest is the fact that they form in their course small neurofibrillar corpusclar dissolutions in places as shown with distinctness in Fig. 3. These corpuscles are much smaller and fewer than the neurofibrillar terminal plates in terminations Type I in adults, but it would be premature to conclude that they are infantile formations of such plates. The correspondence is not conclusive. But it is of interest to note that fibres showing tendency to form similar neurofibrillar dissolutions are abundantly developed in aortic nerve in the embryonic stage, as well as by adults.

As may be seen in Fig. 3 and Fig. 4, the sensory nerve fibres penetrating the media come to repeated anastomosis and then branch out again. Their course is very irregular, showing no definite direction. This fact is also very characteristic to these sensory nerve fibres. Besides, these fibres

Fig. 3

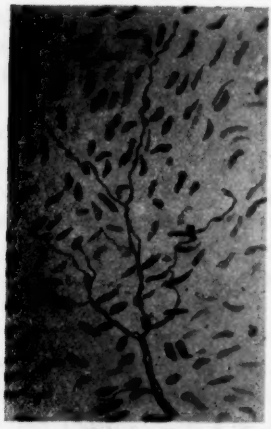

Fig. 4

Fig. 3. Sensory arborized termination originated in aortic nerve found in media of ventral wall of arcus aortae of 8th month embryo. Remarkable change in size of terminal fibres, especially fibrillar corpusclar dissolutions of varying size are seen here and there. Same staining. X 450, reduced to 2/5.

Fig. 4. Sensory termination of irregular form originated in aortic nerve, found in the media of medial wall of ascending aorta in 4th month human embryo. Peculiar formation of branching and anastomosis between terminal fibres especially noteworthy. Same staining, X 450, reduced to 1/2.

have Schwann's nuclei or smaller specific nuclei presumably of the same nature as the former at their terminal parts.

It is certainly doubtful whether the above described sensory terminations formed in the media represent the infantile type of terminations Type I in adults. And, as we can observe almost no blood vessel penetrating the media, the formation of terminations Type II in adults also is not found in embryonic stages. It may be that they are formed gradually after birth, but I will postpone discussion on such points till my concluding remarks.

I have detailed my study on the terminal formation of the aortic nerve in the above, as far as my observation goes, but Muratori[7] and Palme[10] have previously discovered something that may be looked upon as termination of aortic nerve in the media of arcus aortae in human embryos, as mentioned at the outset. However, as the observation of Palme is based upon a single illustration, which was far from well-done, and Muratori's on another solitary picture under low magnificantion, their terminal formation lacks distinctness in description. It may be

concluded that their failure is due to inadequate silver-staining method at their command.

Vegetative nerve fibres are present in the externa of aorta in a large quantity. These consist of thin non-medullated fibres and are easily distinguishable histologically from thick sensory fibres. Alike all other numerous achievements on the subject of this laboratory, the termination of vegetative fibres is shown already in 4th month embryo by the terminalreticulum (Stöhr). This is distinctively formed in the externa of aorta, especially along the nutritive vessels found therein, and often penetrates further into the media. No such terminalreticulum seems to have been proved in the media of adults, and it may be presumed that it is limited to the embryonic stage in existence.

In 1935, Muratori[7] in his study on rabbits found sensory fibres in the truncus brachiocephalicus at the point where it bifurcates into a. subclavia dextra and a. carotis comm. dex. in the interspace, ending in the externa. In my study with human embryos, I found sensory fibres running into the externa and thence into the media at the incipient parts of a. carotis comm. sin. and truncus brachiocephalicus, to terminate after irregular branching, some branches penetrating even into the intima, as shown in Fig. 5. As the terminal formation and the nature of the terminal fibres are similar to those seen in the media of aorta, it is presumed that these sensory terminations also originate in aortic nerve.

Seto has quite recently found sensory terminations in the muscular layer of a. carotis comm. sin. and truncus brachiocephalicus in adult dog, but this may be a phenomenon seen only in similar animals, for in view of the observations made on arcus aortae in human adult, it is more than doubtful if such terminations are ever found after birth in man.

It has been thus ascertained that terminations of aortic nerve exist by human embryo in aorta ascendens and incipient parts of truncus brachiocephalicus and a. carotis comm. sin. Furthermore, I succeeded in confirming the existence of sensory terminations in a. pulmonalis, similarly penetrating into the media and sometimes further into the intima. On this problem, some investigators touched upon the existence of sensory terminations in the pulmonary artery chiefly in animal fetus, but no dependable illustration seems to have been adduced to support their remarks.

These sensory terminations formed in the pulmonary artery originate in the aortic nerve running between aorta ascendens and a. pulmonalis and are found mostly in its dorsal wall facing the ascending aorta. Their terminal formation is almost identical with that found in the media of aorta, as shown in Fig. 6.

Now, these sensory terminations found in the muscular layer of the

Fig. 5

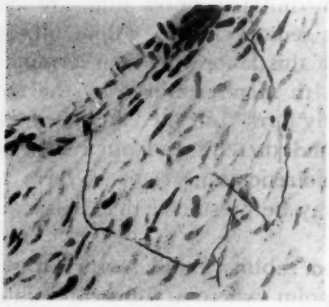

Fig. 6

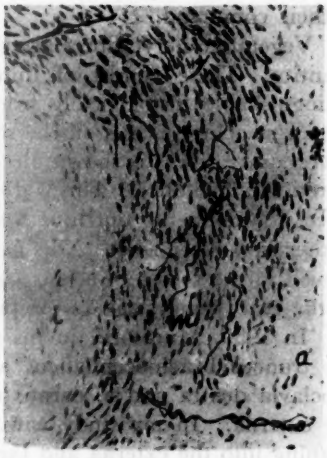

Fig. 5. Sensory termination originated in aortic nerve distributing with very irregular arrangement in the media and intima of the incipient part of a. carotis comm. sin. of 8th month embryo. Same staining. X 450, reduced to 2/5.

Fig. 6. A number of sensory terminations originated in aortic nerve formed in the media of stem pulmonary artery of 4th month embryo. a externa; m media; i intima. Same staining. X 450, reduced to 1/2.

Fig. 7

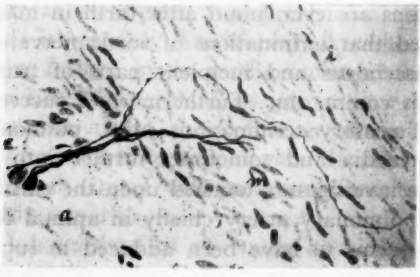

Fig. 8

Fig. 7. Simple sensory termination originated in aortic nerve ending in media of stem pulmonary artery of 4th month embryo. a externa; m media; i initma. Same staining. X 450, reduced to 1/2.

Fig. 8. Comparatively complex branched termination of aortic nerve fibre ending in media of stem pulmonary artery of 4th month embryo. All terminal fibres show marked change in size. Same staining. X 450, reduced to 1/2.

pulmonary artery, in 4th month embryo, are represented by very irregular branched terminations composed of peculiar fibres showing considerable change in thickness and corpusclar fibrillar dissolutions in places, as shown in Fig. 7 and Fig. 8. In these cases also, they are found accompanying Schwann's nuclei and smaller special nuclei and being arranged chiefly circularly in the muscular layer. On the contrary, in 6th to 8th month of embryonic life, these terminations seem to undergo a degeneration, and in 8th month embryo the sensory terminations in the muscular layer decrease almost to nil, existence of extremely simple branched terminations being observed only in the externa covering the media. Consequently, it is supposed that the sensory terminations in adult pulmonary artery show extreme degeneration, so that almost none of them remains to be seen. It is an important problem to be clarified in the future.

Discussion

The sensory terminations of aortic nerve in human embryos have benn witnessed not only in the lower edge of arcus aortae, as in adults, but also in the entire wall thereof, the ascending aorta, the incipient parts of truncus brachiocephalicus and a. carotis comm. sin., and a. pulmonalis, in particular, in the media. However, they are considerably different from those characteristic two types found in arcus aortae and sinus caroticus in adults and connected with reduction of blood pressure. Furthermore, they are in very conspicuous formation in 4th month of embryonic life, but degenerate in the later stage, to become very small in number in 8th month embryo, especially in a. pulmonalis.

So, the problem how the terminations of aoxtic nerve in human adults are formed assumes peculiarly deep interest. As the sensory terminations of aortic nerve formed in the muscular layer in the embryonic life are all represented by undetermined terminations composed of nerve elements of similar nature and showing almost similar form, wherever they may be found, they must be looked upon as special terminations morphologically different from the terminations Type I and Type II found in adults.

Now, what is the process of transition of these special terminations in embryos to the two types of terminations in adults? I am inclined to explain the course of change as follows, to match the observation described hereunder. According to Seto[16] and Yamada,[17] in the embryonic stage, the epithelium of glans penis and glans clitoridis and the epithelium of the inner plate of praeputium unite to form a common epithelium, where are found many complex terminations of intraepithelial nerve fibres showing very peculiar forms. But these terminations are found degenerated and decreased in both the epithelia which separate after birth and

disappear altogether in due time. In the embryonic stage, no formation of corpusclar terminations, such as of genital nerve bodies, is ever found in the connective tissue beneath the epithelium, and it may be assumed that there are some stem fibres of the intraepithelial fibres stated above which change into genital nerve bodies after birth. That is, the complex terminations of the intraepithelial fibres disappear after birth and the tips of the stem fibres change to form new sensory terminations, including genital bodies.

The terminations of the aortic nerve are also formed, in the embryonic stage, in the media of the major arteries mentioned above, but likewise come to complete extinction after birth, and the majority of the stem fibres becomes limited in existence to the connective tissue of the externa of the lower edge of arcus aortae. From the tips of these remnant fibres, terminations Type I and Type II, are newly generated. However, more minute study must be gone through, before it may be decided whether the terminations found in the media of truncus brachiocephalicus, a. carotis comm. sin. and a. pulmonalis come to complete degeneration in adults.

In conclusion, I may venture my opinion on the reason why the sensory fibres of aortic nerve are extended over a much larger area in the embryo than in adult, and penetrate even into the intima. The reason of such an arrangement persisting in the embryo seems to lie in the physiological condition that it is necessary in the fetal life to keep up prompt adaptability of blood pressure to fluctuations of the circulation of the maternal body.

Summary

Sensory terminations of aortic nerve acting reducingly on blood pressure are in the fetal life dispersed over a large area centered on the lower edge of arcus aortae i.e. over the entire of arcus aortae, aorta ascendens, the emergent parts of truncus brachiocephalicus and a. carotis comm. sin. as well as a. pulmonalis. Their development, however, is more remarkable in the earlier half of the fetal life, coming into degenerative change in the later stage, especially in a. pulmonalis.

Sensory terminations of aortic nerve found in arcus aortae and aorta ascendens, widely unlike their counterpart in adult, are formed along the smooth muscle fibres of the media and of uncomparably simpler formation. But in quantity, they surpass by far such terminations in adult. Consequently, these cannot be looked upon as corresponding to the Type I terminations in adult.

As almost no blood vessel is found penetrating the media, no sensory terminations corresponding to the Type II terminations in adult are formed in fetal stage. They are likely to be formed gradually after birth.

Sensory terminations found in the incipient parts of truncus brachiocephalicus and a. carotis comm. sin. of human embryo pass from the externa into the media and sometimes even the intima and their terminal formation is similar to those seen in aorta. Consequently, they must undoubtedly have their origin in aortic nerve.

In the pulmonary artery also is observed the existence of sensory terminations of similar conformation. They are discovered in a large quantity in 4th month embryo, but in 8th month embryo they are found degenerated to a large extent and remaining as a few simple branched terminations in the externa.

The above described terminations of aortic nerve in embryo are believed to change over into the Type I and Type II terminations after birth. The process of transition, I presume, consists in that the terminations in the media so gradually degenerate and disappear, that the majority of their stem fibres retrocedes to be only limited to the externa of the lower edge of arcus aortae, and then the tips of stem fibres newly develop peculiar terminations coming under the Types I and II.

The reason why the sensory fibres of aortic nerve extended over a large area of major arteries terminate in the media thereof is thought to lie in the fact that the circulation activity in the embryo has to keep up incessant and extremely flexible adaptation to the fluctuation in the flow of blood in the maternal arteries.

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Innervation especially Sensory Innervation of Facies Inferior Linguae in Human Adult

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Not a few studies on the innervation of tongue have been reported, for example, by Civalleri,[1] Ábrahám[2] Szymonowicz[3] and others on mammalian tongue and by Ceccherelli[4], Kadota,[5] Nakayama,[6] Inazawa[7] and others on human tongue. However, all of these studies are concerned with the dorsum linguae and radix linguae, while I could not find one single study on the innervation of facies inferior linguae. In face of such a situation, I undertook to enter upon this unexplored field, and taking human tongues as my material, which were first fixed in 10% neutral formol, cut into 40 μ frozen sections, and then stained with Seto's silver impregnation, I subjected the series of many beautiful slides thus obtained to a minute microscopic examination, and arrived at the following results.

Individual Views

1. Histological description of facies inferior linguae

I will preface my description of the innervation with a histological picture of the under surface of human tongue, since this subject seems to have been hitherto too much neglected. In the well-known hand- or textbooks, both Japanese and foreign, nothing is given beyond a very cursory description thereon, such as " The under side of the tongue has no proper lingual papillae, but is covered by a smooth mucous membrane containing common papillae." My detailed observation has prompted me to propose addtion of several new informations on the subject, and these facts especially seem to stand in close relation to the distribution of sensory fibres here.

The mucous membrane of facies inferior linguae is surfaced, under microscopic observation also, generally smooth and even, lacking such protruding papillae as tongue papillae, and is covered with non-conrified stratified flat epithelium. The formation of common papillae for this epithelium originating in the subepithelial connective tissue of the lamina

prppria is rather marked, and their development shows a relatively considerable difference according to the location. If the rule established by numerous achievements obtained in this laboratory that the development of such papillae indicates the proportional development of sensory fibres is applied here, the development of the sensory fibres found in the mucous membrane of the facies inferior linguae should be abreast the development of the papillae formation—a very interesting phenomenon.

Now, at the foremost part of facies inferior linguae, that is, in the formost 1/4 of the area, except at the marginal parts, the epithelium is incomparably smaller than in dorsum linguae in height. The papillae formed by the penetration of lamina propria into the epithelium are in general of regular elongated shape, but some take the form of more or less thick cylinders. In the marginal parts, the epithelium is much higher, and interestingly enough, I could observe many large papillae having small secondary papillae, or even formation of papillae similar to the papillae fungiformes found on dorsum linguae. These fungiform papillae generally lack the groove surrounding them, but some of them even have a depression around them that gives them a structure resembling the fungiform papillae on dorsum linguae. In the low epithelium of the surface taste-bunds are observable, and mostly in plural number. In the parts nearer the median line, the epithelium often thickens considerably, and rather large papillae are observed in places.

The epithelium and the papillae formation in the next quarter area are not much different from the preceding. Accordingly, also here in the marginal parts the epithelium develops strongly, the large papillae with secondary papillae and my so-called taste-bud papillae being also formed remarkably. In the third quarter area of facies inferior linguae, where plica fimbriata makes conspicuous appearance, the epithelium is generally thinner than in the preceding two quarter areas and the papillae become smilarly lower in height. But in the marginal parts, the epithelium is of considerable thickness yet and the papillae formation is rather marked.

In the hindermost quarter area, the epithelium becomes thinner and the formation of the papillae weaker, except in the marginal parts, where the epithelium is kept rather thick yet. At the extreme rear of the tongue, the epithelium and the papillae are of weakest development. Also the epithelium of the plica fimbraiata is very thin and the development of papillae proportionally weak.

To my surprise, in the entire facies inferior linguae I found very peculiarly formed papillae provided with taste-buds. I know no literature in the past touching this fact, and I will try to give some further informations thereon in the following. These my so-called taste-bud papillae

are of the same category as those found in the marginal parts described above, the taste-buds being always formed in the thin stratified flat epithelium surrounding the tip of the papillae. This formation of the taste-buds is proved evidently also in pharynx (Sasaki,[8] Matsumoto[9]), larynx (Sato),[10] and radix linguae (Inazawa)[7] in the thin stratified flat epithelium, which is not accompanied by the formation of papillae. That is to say, that taste-buds are formed, regardless of having papillae or not, in the thin non-cornified stratified flat epithelium. Taste-bud papillae in facies inferior linguae are in general large in size and very often provided with lymphocytes gathering, similar to that of the large papillae with taste-buds in palatum molle described by Sakai,[11] the taste-buds being in most cases in plural number. Such special papillae are found also in the margin of plica fimbriata, though in a small number.

Thus, I succeeded in proving the existence of the taste-bud papillae in the facies inferior linguae, not only at the marginal parts but also in almost all other parts. It seems that the existence of the taste-buds has been hitherto limited to the papillae circumvallatae and papillae foliatae of dorsum linguae and the epiglottis. But recent researches have ascertained their existence in many other parts, such as the fungiform papillae (Nakayama),[6] the peculiar large papillae of the palatum molle (Sakai),[11] in the epithelium of radix linguae (Inazawa)[7] and pharynx (Sato)[10] and also in the lower part of the plica vocalis and the pars intercartilaginea of glottis (Matsumoto).[9] My study resulted in a further extension of the area in which the existence of the taste-buds has been proved.

2. Plexus and vegetative fibres in facies inferior linguae

Of the nerves for the facies inferior linguae, sensory fibres originate in r. linguae of n. glossopharyngicus, n. lingualis from n. mandibularis of n. trigeminus and chorda tympani from n. intermedius, and motor fibres in n. hypoglossus. The vegetative, especially the sympathetic, fibres seem to come from the ganglion cervicale craniale. These nerve elements run into the tongue from the hinder-lateral side, and only the sensory and vegetative fibres reach the mucous membrane, while the motor nerves disappear in the muscular layer. The former two kinds of nerve elements appear as nerve bundles of varying sizes in tunica submucosa, run into the loosemeshed plexus spreading out in a plane, and finally penetrate lamina propria, to take part in forming the dencemeshed plexus spreading out flatly.

The sensory fibres in plexus are represented by medullated fibres containing thick axon cylinder, while the vegetative fibres consist of fine non-medullated fibres, so the histological distinction of the both fibres

is very easy. The termination of vegetative fibres is represented by Stöhr's terminalreticulum also here in the subepithelial connective tissue, as in other parts of the body. It never ends free, is distributed widely and stands in tactile innervation for all of the tissue cells existing here.

3. Distribution of sensory nerve fibres

It is not difficult to surmise that the sensory fibres in the mucous membrane of facies inferior linguae is developed far from poor, seeing that the mucous membrane is provided with well-developed papillae, and also that taste-buds papillae are formed in many parts. In particular, in proportion of the existence of large papillae and taste-buds papillae in the marginal parts, the development of the sensory nerve terminations must be remarkable. The vicinity of the median line is also provided with large papillae, so the sensory terminations are also in good development here. As we go backward, the epithelium becomes thinner, the papillae formation weaker and the sensory fibres become poorer accordingly.

The terminal mode of the sensory fibres in the facies inferior linguae is very various, but may be classified into the following types: 1. Simple branched and unbranched terminations; 2. Complex branched terminations; 3. Capsulated and non-capsulated glomerular terminations; 4. Special terminations for taste-buds. Since these terminations are mainly formed in the papillae and their complexity corresponds the degree of development of the papillae, I feel justified in taking the kinds of concerned papillae as headings, in detailing the terminations of all types.

Sensory terminations in large papillae with secondary papillae.

The large papillae are found mainly in the marginal parts of facies inferior linguae, and also often near the median line. Their papillar trunk and the formation of the secondary papillae are very similar to those of the papillae filiformes in dorsum linguae, except in that they do not protrude from the surface together with the covering epithelium and in that the epithelium has no corneal layer, as do the papillae filiformes. That is, as these papillae are covered by the non-cornified stratified flat epithelium in common with the neighbouring papillae, the surface of the common epithelium is kept always nearly flat and smooth.

It may be deduced from the histology of these large papillae described above, that the distribution of nerve elements is similar to that in the papillae filiformes, but against all such anticipation, the nerve elements penetrating them, especially, the sensory fibres of varying sizes originating in the plexus formed at the base of the papillar trunk are found incomparably fewer than in the papillae filiformes. But the quantity is only small in comparison with the case of the filiform papillae, which are provided with unusually well-developed sensory fibres (Nakayama),[6] and generally

speaking, the development of sensory fibres is not at all poor here.

The sensory terminations found in these papillae can be represented by all the above mentioned types of terminations, but special mention must be made of the capsulated glomerular and the branched or plexus-like terminations arranged very irregularly. But these are all far weaker in number and scale in comparison with those in papillae filiformes (Nakayama).[6] For example, glomerular terminations are found in usual only one or two in one large papilla, the cases of three or four such terminations in it as shown in Fig. 1 being of rare occurrence indeed.

As shown in Fig. 1, and more clearly in Fig. 2, the glomerular terminations originate in one or two medullated fibres, which, after losing the myelin, divide into only a few branches, which show peculiar change in size and a winding course and terminate in a comparatively simple glomerular arrangement in the inner bulb surrounded by a thin connective tissue capsule. The inner bulb contains a number of oval-shaped special nuclei.

Plexus-like terminations are formed of two or three medullated fibres, which, upon losing the myelin, divide into two or three branches. These terminal branches show change in size in places and run an extremely irregular winding course, to show a plexus-like arrangement in total appearance. Very often, fine vegetative fibres are seen therein, forming vegetative terminalreticulum, as shown in Fig. 3 and Fig. 4.

Sensory terminations in small papillae. Small papillae are formed in all of the surface of facies inferior linguae. In its rear parts, they become even lesser in size. The sensory fibres for these small paillae are incomparably smaller in quantity than those for the above described large papillae. The weaker the formation of the papillae, as in the hinder parts, the less the quantity of nerve fibres therein. There are even many petty papillae where no sensory fibre enters at all. In the papillae where sensory fibres are found, their terminal mode is in gneral very simple and represented by the unbranched terminations having no subbranches (Fig. 5) or simple branched terminations consisting of a few branches, as seen in Fig. 6.

However, as shown in Figs. 7, 8 and 9, formation of corpusclar terminations is not always rare. These are found in the somewhat larger type of small papillae, and represented by the non-capsulated glomerular terminations in most cases. They are quite similar to the corresponding terminations in the large papillae described above, and generally consist of a large medullated fibre, which, after losing its myelin and showing the specific change in size and the peculiar winding course with a few branches, terminates in a comparatively simple glomerular terminal formation. In the glomerular bodies, there are found many special nuclei. In rare cases, the glomerular bodies are formed in plural number in one small

Fig. 1

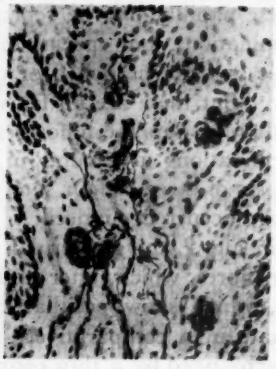

Fig. 2

Fig. 3

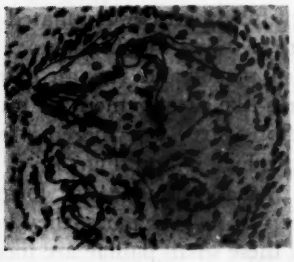

Fig. 4

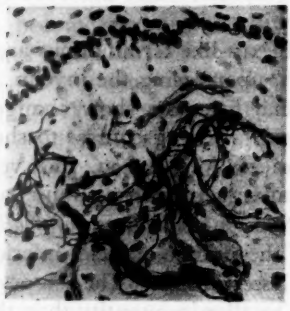

Fig. 1. Distribution of sensory fibres in a large papilla with many secondary papillae found near the median line in facies inferior linguae of adult. 3 simple glomerular terminations are formed conspicuously. Seto's silver method, X 200, reduced to 1/2.

Fig. 2. 2 capsulated glomerular terminations found in a large papilla in the margin of middle part of facies inferior linguae of adult. Same staining, X 500, reduced to 2/5.

Fig. 3. Plexus-like termination in a large papilla found in the margin of foremost part of facies inferior linguae of adult. Same staining, X 320, reduced to 1/2.

Fig. 4. Plexus-like termination formed by thick sensory fibres commingled with minute vegetative fibres, part of which forms the terminalreticulum, found in a large papilla in the margin of middle part of facies inferior linguae of adult. Same staining, X 500, reduced to 2/5.

papilla, for example, in Fig. 8, where three small bodies are found in a small papilla.

Sensory terminations in taste-bud papillae. The taste-bud papillae found in facies inferior linguae are formed stronger in the lateral parts than in

Fig. 5

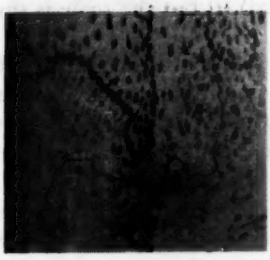

Fig. 6

Fig. 7

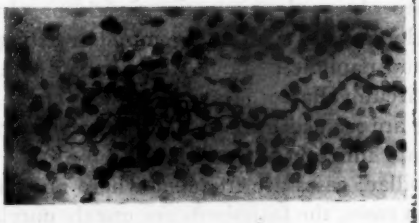

Fig. 8

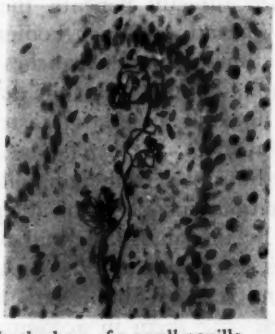

Fig. 5. Unbranched termination formed in the base of a small papilla in the middle part of facies inferior linguae of adult. Same staining, X 400, reduced to 2/5.

Fig. 6. Simple branched termination found in a small papilla in the foremost part of facies inferior linguae of adult. Same staining, X 400, reduced to 2/5.

Fig. 7. Non-capsulated glomerular termination formed in the tip of a small papilla in the middle part of facies inferior linguae of adult. Same staining, X 500, reduced to 3/5.

Fig. 8. 3 non-capsulated glomerular terminations formed in a small pailla in the middle part of facies inferior linguae of adult. Same staining, X 500, reduced to 1/2.

the more medial parts, but in comparison with the large paillae with taste-buds in palatum molle (Sakai),[11] they are smaller in scale. Concerned with the innervation of these taste-bud papillae, the conspicuous plexus-formation consisting of fine nerve bundles is first noticeable at the

basis of the larger papillae (Fig. 10). This plexus is composed of thick medullated sensory fibres and many fine non-medullated fibres, all running further into the papillar trunk. This situation much resembles that in the taste-bud papillae of palatum molle (Sakai),[11] but the scale is smaller than of the latter.

These sensory fibres mostly penetrate the connective tissue of the papillar trunk, divide into a few branches and finally pass into simple arborized terminations or often into undetermined terminations showing no typical form. Among these terminations, a great number of non-medullated fibres is seen commingled and spreading out net-wise. As these on-medullated fibres in the end come to form Stöhr's terminalreticulum, as shown in Fig. 10, they should be looked upon to belong to the vegetative fibres.

Some of the sensory fibres in the large taste-bud papillae, above all, the thick ones often pass into conspicuous corpusclar terminations, especially into non-capsulated glomerular terminations. They are usually formed directly beneath the epithelium or in the secondary papillae. The formation of such bodies can be observed also under the taste-buds. Sometimes, minute branches are observed penetrating inside or outside of the taste-buds. But the development of such intragemmal or extragemmal fibres in these large taste-bud papillae is much weaker than by those typical ones in the paillae circumvallatae (Nakayama).[1] Then, the taste-buds here in these papillae are seemed to be more or less degenerated formations incapable of adequate function.

By the small taste-bud papillae, cases are frequent where no sensory fibres enter them at all. In such cases, the taste-buds are utterly unrelated to any sensory fibre. But, also here are seen not rarely sensory fibres penetrating them in accompaniment of non-medullated fibres, and they can also form beneath the taste-buds comparatively typical non-capsulated glomerular terminations (Fig. 11 and Fig. 12), or they can send out minute branches into the taste-buds. But, as in these small taste-bud papillae the sensory fibres are usually very small in number, and such intragemmal fibres are of rarest occurrence, they must be deemed as of even further degenerated formation, accordingly much weaker than those in the large taste bud papillae described above in function.

Summary

The epithelium of facies inferior linguae mostly is much thinner than that of dorsum linguae, the formation of the papillae being correspondingly weaker, especially as the rear parts are approached. But, in the marginal parts the epithelium is thicker, and large papillae provided with secondary papillae or especially with taste-buds are observable accordingly. As

Fig. 9

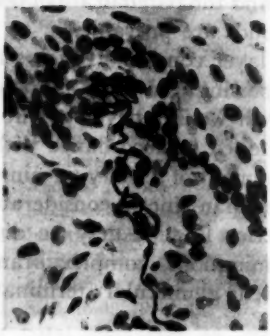

Fig. 10

Fig. 11

Fig. 12

Fig. 9. Non-capasulated glomerular termination formed in a small papilla in the foremost part of facies inferior linguae. Same staining, X 500, reduced to 3/5.

Fig. 10. Nerve distribution in a large taste-buds papilla in the marign of the foremost part of facies inferior linguae of adult. Thick fibres are sensory, fine fibres vegetative, which in part form the terminalreticulum. Same staining, X 280, reduced to 2/5.

Fig. 11. Conspicuous glomerular termination formed in a small taste-bud papilla in the foremost part near the median line of facies inferior linguae of adult. Same staining, X 500, reduced to 1/2.

Fig. 12. Ditto.

the epithelium of the vicinity of the median line is thick too, comparatively large papillae or taste-buds papillae can be seen also here. However, all of the papillae seen in facies inferior linguae even do not admit protrusion of epithelium over the surface, in contradistinction to the papillae in dorsum

linguae, and the epithelial surface is kept always smooth and even. The epithelium of the plica fimbraiata is thinnest and the papillae are of poorest development, yet a very few of small taste-buds are provable.

The plexus formed in the subepithelial layer consists of thick sensory and fine vegetative fibres, the latter finally passing into the Stöhr's terminalreticulum. The sensory fibres are of much weaker development than in dorsum linguae, but are rather well-developed in accordance with the development of the papillae. So, the development of sensory terminations in marginal parts and the vicinity of the median line is considerable.

The sensory terminations in facies inferior linguae may be divided into simple unbranched and branched terminations, complex branched terminations, non-capsulated and capsulated glomerular terminations, and special terminations for taste-buds.

Of the sensory terminations found in the large papillae without taste-buds, glomerular terminations, especially capsulated ones, and plexus-like terminations may be mentioned as of special interest, though they are much lower numerically than in dorsum linguae. The former consist of thick sensory fibres, which after losing the myelin, show peculiar change in size and a winding course and terminate in simple glomerular arrangement. They are in general capsulated and provided with numerous special nuclei in the inner bulb. Plexus-like terminations are composed of two or three thick sensory fibres dividing into two or three branches, which show the irregular plexus-like terminal arrangement.

The development of sensory fibres for the samll papillae is very weak in comparison with the above, and many of them even lack sensory fibres. And in small papillae where sensory fibres enter, their terminal mode is simple, mostly represented by unbranched or simple branched terminations. However, there are found not rarely corpusclar, especially simple non-capsulated glomerular terminations, and two or three such bodies can be found in one small papilla, though in a small quantity.

In the large taste-bud papillae, plexus is formed at the base, and the sensory fibres penetrate the papillae, being accompanied by vegetative fibres, mostly to pass into simple arborized or undetermined terminations, but partly into corpusclar, especially into non-capsulated glomerular terminations. These bodies are frequently formed just beneath the taste-buds, sometimes sending out intra- and extragemmal fibres.

The small taste-bud papillae are very often devoid of sensory fibres, but sometimes sensory fibres run into them with vegetative fibres, to pass into non-capsulated glomerular terminations in the tip of the papillae, more rarely sending out intragemmal fibres.

As detailed above, since the morphological development of sensory fibres for the taste-buds found in facies inferior linguae is incomparably

weaker than in the papillae circumvallatae, it may be assumed that they are also functionally much degenerated ones.

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Innervation especially Sensory Innervation of Dog Tongue

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Many histological studies[1),2),3)] on the innervation of mammalian tongues have been reported and praiseworthy results achieved histherto, but much seems yet left unexplored, and in particular, many questions are still waiting solution in connection with dog tongue. Recently, much licht has been thrown on the subject of innervation of human tongue by the minute studies by Kumagawa,[4)] Nakayama,[5)] Inazawa[6)] and Ohgaki[7)] of this laboratory. So, I took the tongues of dog as my material, and through a thoroughgoing histological study on its innervation, especially sensory innervation undertook to elucidate its biological meaning, in comparison with the innervation of adult human tongue.

The materials were taken from fresh adult dog tongues, which were fixed for a long time in 10% neutral formol, cut into 40 μ frontal frozen sections, and stained with Seto's silver method in use in this laboratory. The series of beautiful stained preparations I subjected to a minute microscopic scrutiny and succeeded in arriving at many new informations as described in the following.

Individual Views

As is well known, the motor nerves for the tongue come from n. hypoglossus, while the sensory nerves from n. lingualis of n. mandibularis of n. trigeminus, n. glossopharyngicus as well as chorda tympani of n. intermedius and rr. pharyngici of n. vagus. The vegetative nerves, especially sympathetic nerves come from ganglion cervicale craniale. These nerve bundles enter from the outer side of the radix linguae into the tongue muscles, and gradually dividing into smaller bundles, run to the periphery, to pass into crude nerve plexus. In the course, they contain the undermentioned multipolar nerve cell groups. Thick medullated motor fibres all end in the striated muscle fibres.

The small nerve bundles entering the subepithelial connective tissue

through the muscle layer consist of thick medullated sensory and non-medullated vegetative fibres, and pass into plexus, which is developed far lower than in human tongue. Though in the base of the papillae foliatae and circumvallatae conspicuous basal plexus are formed, otherwise, almost nothing that may be called as plexus could be found in formation. There are besides such vegetative fibres, which, running along the blood vessels, form so-called vessel wall plexus, as is the case in human tongue. They come into frequent anastomosis with the non-medullated fibres in the above mentioned nerves.

1. Innervation of dog tongue muscles

The terminal mode of motor fibres for the tongue muscle in dog is generally speaking similar to that of human tongue (Kumagawa).[4] So, the medullated stem fibres, as they approach the terminal area, in most cases without losing the myelin, divide into 2 or 3 branches, then lose the myelin and pass into very minute 2 to 5 terminal branches, each of which terminates sharply or with a fibrillar plate or a small ring in minutely granulated motor end-plate containing numerous round special nuclei (Fig. 1).

About the existence of Boeke's[8] so-called accessory fibres of sympathetic nature, I am very much in doubt, as were Cajal,[9] Hirano[10] and Kumagawa,[4] for I have found not rarely the existence of minute fibres dividing from motor nerve fibres to end freely in the motor end-plate, and sympathetic fibres terminate never freely.

As Nakayama[5] proved the existence of rather well-developed muscle spindles in human tongue, I made a strenuous research for such formation in dog tongue but in vain. However, as Hatano[11] reports existence of muscle spindles in the foremost part of dog tongue, it may be that a very limited formation of such spindles is possible.

2. Distribution of vegetative nerves in dog tongue

I frequently found existence of groups of 2 to 10 nerve cells in or near the small nerve bundles penetrating the tongue muscles. These cells are generally impervious to silver impregnation and also the nerve processes stain badly, but whenever the silver impregnation is well executed, the multipolarity of the nerve cells is proved clearly, and there is no doubt that they are of sympathetic nature (Fig. 2). Nerve cells are also observed accompanying the small nerves running between the lobules of serous glands in the deeper layer of papillae folitatae and circumvallatae. These ganglion cells as well as the Remak's hemi-ganglion cells in the basal plexus of the above two kinds of papillae and the nerve cells sporadically found in the tela submucosa should belong to the sympathetic system

Fig. 1

Fig. 2

Fig. 1. Motor nerve terminations in dog tongue muscle. Seto's silver impregnation. X 1000, reduced to 1/2.

Fig. 2. Multipolar nerve cell group found in the intermuscular connective tissue in dog tongue. Same staining, X 500, reduced to 1/2.

mentioned above, according to my views, which are in concurrence in the views of Nakayama[5] and Inazawa[6] on human tongue. In short, in these cells, especially the development of nerve processes is extremely bad, somewhat unlike those in the common sympathetic ganglia, and as this fact shows the degenerated character of these cells, they are accordingly deemed as inferior in their functional efficiency.

The termination of the vegetative fibres running in the tongue is always represented by the terminalreticulum (Stöhr),[12] either in gland parenchyma, in muscle layer or in subepithelial connective tissue, as is the case with human counterparts. This terminalreticulum stands in tactile supply for all controlled cells, including gland cells, and never penetrates the cell bodies.

3. Sensory supply of dog tongue

The sensory terminations found in the dog tongue are mainly formed in the mucous membrane. The development of the sensory fibres is incomparably much less than in human tongue. That is, at the base of foliate and circumvallate papillae, the development of basal plexus is rather good, but otherwise, the plexus formation in tela submucosa and lamina propria is very poor and the sensory fibres in it are very limited in quantity.

Now, the sensory supply of all kinds of papillae is described in detail in each of following chapters, and I must dwell shortly therein also upon the fine structure of every papilla, since these paipllae show various peculiar constructions rather dissimilar to the human tongue papillae, and this fact affects closely the sensory innervation.

Filiform papillae. The filiform papillae in dog are very different from those in human tongue. They are incomparably smaller in scale, generally of short cylindrical form having only a few secondary papillae, and though the epithelium is cornified, it does not show such a strong corneous plate formation as seen in human tongue. These are rather large in the foremost part of the tongue, but gradually diminish in size as the hinder part is approached. Thus, the filiform papillae in dog are very weak in development and the sensory terminations are correspondingly backward in development (Fig. 3).

The sensory fibres running into the connective tissue trunk of the filiform papillae consist of thick and thin medullated fibres, which are gathered in 2 or 3 fibres, in rarer cases, in 5 or 6, and in accompaniment of fine vegetative fibres, run into the trunk, often as far as the secondary papaillae.

As terminal forms in the filiform papillae, simple unbranched terminations consisting of sensory fibres ending sharply without any branching after lost of the myelin (Fig. 3) and simple branched terminations consisting of 2 or 3 simple branches (Fig. 3 and Fig. 4) may be mentioned.

Fig. 3

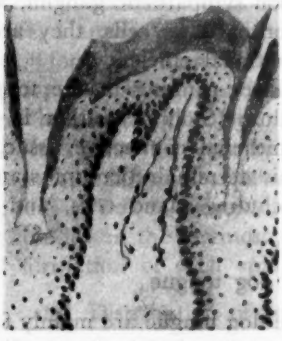

Fig. 4

Fig. 3. An unbranched and a branched termination formed in a filiform papilla in dog tongue. Same staining, X 200, reduced to 2/5.

Fig. 4. Branched termination divided into 3 branches found in a filiform pailla. Dog tongue. Same staining, X 500, reduced to 2/5.

The stem fibres are generally medium sized or thin, but sometimes thick fibres also can be seen, the latter very often showing peculiar change in size and winding course (Fig. 5). No intraepithelial fibres, into which the terminations further pass, are seen running, in all probability because the epithelium is cornified in nature.

Next, the remarkable fact to be mentioned is that there is no where observed in the filiform paillae in dog any complex formed corpusclar

terminations, such as glomerular and genital nerve bodies. When we recollect the view expressed by Seto to the effect that the complex corpusclar terminations are of extremely fine and delicate sensory nature, that is to say, they are the terminal seats of higher sense apperception, it is an interesting fact that there are no such highly evolved terminal apparatus in filiform papillae in dog as seen in human specimen.

Fungiform papillae. Of the fungiform papillae in dog, those in the foremost part of the tongue are comparatively small in size, but those in the hinder part are larger, having conspicuous secondary papillae and containing comparatively numerous taste buds in the covering epithelium.

The innervation of these papillae is very poor in the foremost part, just like in the filiform papillae, but shows somewhat more complexity as the hinder part is approached. For example, plexus resembling the basal plexus found in the circumvallate papillae, but far lower in development than this, can be freqently observed (Fig. 6).

Fig. 5

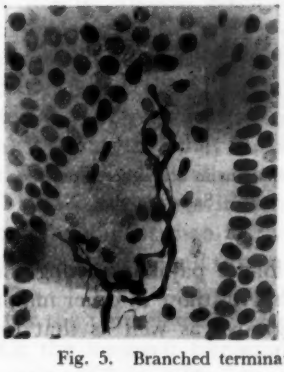

Fig. 6

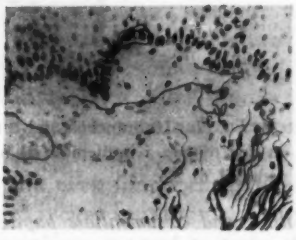

Fig. 5. Branched termination of thick fibre in a filiform papilla. Dog tongue. Same staining. X 1000, reduced to 2/5.

Fig. 6. Distribution of nerve fibres in a fungiform papilla in the hinder part of dorsum linguae in dog. b branched termination; i intraepithelial fibre; t taste bud. Same staining, X 500, reduced to 2/5.

This plexus is composed of rather numerous thin and thick medullated sensory and fine non-medullated fibres. The sensory fibres always run to the periphery of the papillae, to pass into sharply ending simple unbranched or simple branched terminations dividing into 2 or 4 branches. The latter sometimes are represented as plexus-like terminations showing irregular winding arrangement, as shown in Fig. 7.

In the fungiform papillae also, as in the filiform papillae, no corpusclar terminations are found existing, as are so conspicuously formed in those in human tongue. This fact also suggests that the sensory termi-

nations in dog tongue are much lower in functional nature than those in human tongue.

In the fungiform papillae, intraepithelial fibres were clearly observed, which was not the case with filiform papillae. This is apparently due to the fact that the surface of the epithelium here is not cornified, but covered with a thin corneous plate. Their development is, however, very weak, the majority being without any branches. The development of the sensory fibres for the taste buds is also very weak in comparison with those in circumvallate papillae described hereunder. The sensory fibres running toward the taste buds are not at all numerous, sometimes being absent all together. But in rarer cases, fibres are sent furhter out into or around the taste buds as so-called intra- and extragemmal fibres. These, however, are always of the simplest kind, generally ending unbranched (Fig. 6 and Fig. 7).

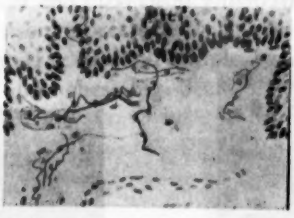

Fig. 7. Unbranched and branched terminations in a fungiform papilla in dog tongue. i intraepithe ial fibre; t taste bud. Same staining, X 500, reduced to 2/5.

Foliate and circumvallate papillae. The foliate papillae in dog show a specially typical development in comparison with those in other mammals. Their epithelium facing the surrounding groove as well as that toward the mouth cavity contain numerous taste buds.

The circumvallate papillae are much fewer in number than those in man, numbering in most cases only 4, and in rare cases, 6 in total. They are considerably different from human ones also microscopically. As shown clearly in Fig. 8, the epithelium facing the mouth cavity, as well as that facing the groove around it, are equally thin, both being provided with taste buds, showing the same arrangement as in human embryo (Nakayama).[5] On the top surface, 1 or 2 grooves of considerable depth are engraved. The epithelium lining these grooves are much thicker than the taste bud epithelium, and no taste buds are seen therein. While the wall epithelium surrounding the circumvallate papillae in man is thin and contains many taste buds, by dog the formation of taste buds therein is very limited, though the epithelium shows the nature of taste bud epithelium yet, so much so that some investigators deny existence of

taste buds in it altogether.

Both the foliate and circumvallate papillae show peculiarity in fine structure of mucous membrane, as shown above, but the minute structure of the deeper layers is not much different from that in man. Under the tunica submucosa, there is striated muscle layers containing strongly developed serous glands, the ducts of which generally open into the basal part of the grooves.

The distribution of the sensory nerve fibres in these two kinds of papillae is as follows. At the base of the papillae, as in man, basal plexus provided with Remak's hemi-ganglion cells is observed, as shown in Fig. 8. The nerve elements therein are composed of thick medullated sensory and finer non-medullated vegetative fibres. The development of the former is considerably strong, not much behind that in man, while that of the latter is somewhat poorer.

The Remak's hemi-ganglion cells are multipolar cells far smaller than the common sympathetic cells, as described above, and are of sympathetic nature without doubt. The medullated fibres penetrating into the papillae from the basal plexus are of course sensory fibres, but some investigators assume that the fine non-medullated fibres also are sensory, especially representing gustatory fibres. However, I look upon these fine fibres as of vegetative nature, as is the case in human tonuge (Nakayama),[5] seeing that they become preterminal fibres, after sundry mutual nerve anastomosis, finally to pass into vegetative terminalreticulum.

The sensory fibres in foliate and circumvallate papillae are in far better development than in the filiform, and even fungiform papillae described above. They all run out of the basal plexus into the papillar trunk, and spread out radially toward the periphery to end mainly directly beneath the epithelium in terminations of various forms.

Of the terminal formations, simple unbranched terminations may be mentioned first. These are found only extremely rarely in these kinds of papillae. Most of the terminations found in these two kinds of papillae are represented by branched terminations showing various forms. They are divided further into simple and complex branched terminations, the latter again into arborized terminations or plexus-like terminations, according to the mode of running course of the branches.

For example, the termination seen at the center of Fig. 9 shows a typical arborized one composed of many branches formed under the epithelium on the top surface of a foliate papilla. In this kind of terminations the fibres show little change in size, tapering off gradually to end mainly sharply. Fig. 10 shows a comparatively complex plexus-like termination formed directly beneath the epithelium of a foliate papilla. In this formation 2–3 stem fibres partake, and the terminal fibres show

Fig. 8

Fig. 9

Fig. 8. Circumvallate papilla and distribution of nerve fibres therein. Dog tongue. Same staining, X 100, reduced to 2/5.

Fig. 9. Branched terminations formed beneath the epithelium of a foliate papilla in dog tongue. A typical arborized termination seen at center. Same staining, X 500, reduced to 2/5.

marked change of size in places. Also the existence of undetermined terminations showing remarkable change in size is frequently observed closely beneath the epithelium. Besides, in these two kinds of papillae, intraepithelial fibres running into the epithelium are found comparatively numerously, though they are mostly of simple unbranched nature.

As described above, in the foliate and circumvallate papillae there are seen many taste buds, the gustatory fibres for which are rather remarkable in development, as detailed hereunder, though not so well-developed as in man.

Also in dog, as in man, the development of gustatory nerve fibres for the taste buds in the circumvallate papillae seems to be somewhat inferior than that in the foliate papillae. In both papillae, it is almost impossible to find such typical glomerular terminations formed directly under the taste buds, as frequently seen in those in man. But under the taste buds of foliate papillae, some smaller sized terminations, that may well be called quasi-glomerular bodies, can be observed, as shown in Fig. 11. On the other hand, under the taste buds of the circumvallate papillae, such complex, quasi-corpusclar terminations are never found. However, it is similar to the case in man, that very simple loop-like terminations (Fig. 12), plexus-like terminations and other kinds of simple branched and unbranched terminations are formed under the taste buds of these both kinds of papillae.

Now, for the taste buds themselves, many intraepithelial fibres originated in all of the terminations formed under the taste buds described

Fig. 10

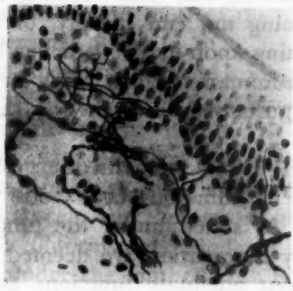

Fig. 11

Fig. 12

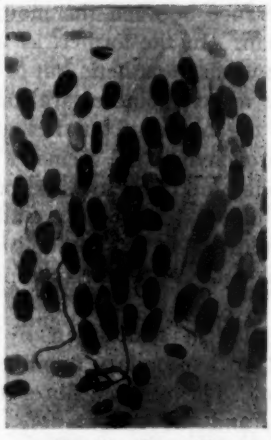

Fig. 13

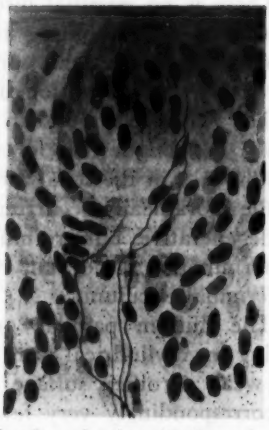

Fig. 10. Plexus-like termination formed directly under the epithelium of a foliate papilla in dog tongue. An intraepithelial fibre is seen at the extreme right. Same staining, X 500, reduced to 2/5.

Fig. 11. Quasi-glomerular termination formed directly under a taste bud in a foliate papilla. Dog tongue. Same staining, X 1000, reduced to 2/5.

Fig. 12. Simple loop-like termination found directly under a taste bud in a circumvallate papilla. Dog tongue. Same staining, X 1000, reduced to 2/5.

Fig. 13. Some intragemmal fibres terminating sharply in a taste bud in a foliate papilla. Dog tongue. Same staining, X 1000, reduced to 2/5.

above very often may be found running into or around the taste buds (intra- or extragemmal fibres), as shown in Fig. 12 and Fig. 13. But as

these fibres generally end unbranched, it may be understood that the development is inferior to that of human counterparts. These intraepithelial fibres, as common intraepithelial fibres, take their running course always inter- and intracellularly, and going up, end sharply, or sometimes bluntly, only in very rare case, forming knob-like or lamellar bodies formed by fibrillar expansion, as seen in human tongue. This fact also suggests the lower development of the gustatory fibres in dog than in man.

So much about the taste buds closely connected with the gustatory nerve fibres. There are furthermore, very numerous taste buds which receive no such sensory supply at all. As stated under the fungiform papillae, such taste buds without sensory innervation are deemed to be more or less degenerated entities, without adequate functions as taste buds.

Summary

There is no essential histological difference between the motor end plates in the tongue of dog and that of man. In the former, as well in the latter, the Boeke's accessory fibres are not of sympathetic but of motor nature. In the canine tongue, few if any muscle spindles are found.

The nerve cells found in many places on the nerve bundles running through the tongue muscles and gland parenchyma, the Remak's hemiganglion cells in the basal plexus in the foliate and cirumvallate papillae and the sporadically occurring nerve cells in tela sumbucosa are all small and degenerative, but are multipolar and must be of sympathetic nature. The termination of vegetative fibres in dog tongue is also represented by the terminalreticulum.

The filiform papillae are much smaller in scale than by man, showing low cylindrical form containing 2 or 3 secondary papillae, with very weak cornified surface of epithelium. The sensory fibres thereto are correspondingly very few in number, and the terminations are in general of simple unbranched or branched type consisting of medium or small size fibres, but frequently of larger fibres showing change of size. No intraepithelial fibres are found to exist. Corpusclar terminations as seen in man also are lacking.

The fungiform papillae are small at the fore part of the tongue but larger at the hinder part, with secondary papillae. In the epithelium taste buds are often observed. The innervation is weak in the fore part, but as we go backward, the nerves become better developed, with frequent formation of basal plexus at the base. The sensory fibres originating in it run into the papillae, to pass into simple branched and unbranched or more complex plexuslike terminations. But also here, no corpuslcar

terminations may be seen. But as the epithelium is covered by the corneous plate, formation of intraepithelial fibres may be observed, though in a weak development. The development of gustatory fibres to the taste buds is also poor, most of them being out of direct supply of intra- and extragemmal fibres.

In the canine foliate and circumvallate papillae, many taste buds exist not only in the epithelium facing the grooves but also in that facing the mouth cavity. But in the epithelium of the wall surrounding the circumvallate papillae, the existence of taste buds is very limited. At the base of these two kinds of papillae, conspicuous formation of basal plexus is seen, and the sensory fibres originating in it are better developed than in filiform and fungiform papillae, forming terminations of various forms under the epithelium. Simple unbranched terminations are little in number, branched terminations being prevalent. Branched terminations are further divided into simple and complex forms, the latter again into arborized and plexus-like terminations showing change in size and peculiar winding course of the fibres.

The development of sensory fibres to the taste buds in these papillae is rather good, but glomerular terminations observable in man cannot be found, only simple quasi-glomerular bodies being formed rarely in the foliate papillae. However, similarly to the human counterparts, loop-like, plexus-like and other simple branched terminations are not lacking under the taste buds here. Intra- and extragemmal fibres often run into or around the taste buds, but these are generally unbranched, ending sharply, never in knob-like nor lamellar bodies. As many of these taste buds are observed without any direct supply of sensory fibres, it may be concluded that the development of the gustatory fibres is much inferior in dog tongue than by human being.

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Isolation and Identification of Methyl Glyoxal

(Pyruvaldehyde or Pyruvic Aldehyde) from Human Milk,* Parts I & II

264th Report of the Peroxidase Reaction
(211th Human Milk Study)

By

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Introduction

This treatise is the detail of the preliminary report[1] which was published in this Journal about several months ago. Since the publication of Arakawa's reaction in 1930[2] from our Laboratory, Prof. Sato and his many collaborators have been studying the significance of Arakawa's reaction for a long time and published a series of papers—more than 120 printed originals in this Journal.

Prof. Sato[3] has always been of the opinion that human milk negative to Arakawa's reaction or Arakawa-negative milk is not only a milk from a thiamine deficient body, but also a *poisonous milk*.

Basing upon Takamatsu's experiment with Arakawa-negative milk producing heart enlarging, for which "methyl gloyoxal" was made responsible, Prof. Sato and T. Takamatsu[4] in 1934 reported the heart enlarging effect of synthetic methyl glyoxal in animal experiment. Sh. Sato[5] of our Laboratory made the occurrence of methyl glyoxal in Arakawa-negative human milk highly probable.

I had wanted very much to attack the problem myself, because Prof. Sato[3] had used to tell me always that Arakawa-negative milk contained a poison or poisons, and that the poison could never be pyruvic acid according to his experiment,[6] and further that it must in all probability be methyl glyoxal, though he had not excluded other glyoxals. But

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however hard I might try, I was at first unable to find methyl glyoxal in human milk at all, so that I almost gave up trying to identify it, but began to think what kind of chemical constituent there could be to act as a poison in human milk. Finally I found out that acetaldehyde in human milk had been the disturbing substance—the very substance disturbing in the identification of methyl glyoxal, and at last I was able to succeed in isolating and identifying methyl glyoxal in human milk as 2,4-dinitrophenylosazone (Cf. Part I & Table I).[1]

Later I was able to isolate and identify it as *p*-nitrophenylhydrazone too, though the identification by way of this osazone was not so accurate a method as that of 2,4-dinitrophenylhydrazone (Cf. Part II & Table I).[1] Further I made the most of paper partition chromatography and was again able to ascertain the very occurrence of methyl glyoxal in human milk, while to my great surprise pyruvic acid could never be identified by paper partition chromatography.

Part I

Isolation and identification of methyl glyoxal from human milk as 2,4-dinitrophenylhydrazone

Methyl glyoxal was once reported as an intermediary product of carbohydrate fermentation and a decomposition product by bacteria, but the identification of it in human materials was rather doubtful and not definite.

Prof. A. Sato[7] in 1931 showed the following expression concerning the significance of Arakawa's reaction.

Human milk negative to Arakawa's reaction=human milk—vitamin $B_1+\alpha$ (Here α means a poison or poisons.)

This was later what was experimentally shown by Takamatsu,[4] Asakura,[8] and Asakura and Osako.[9] And in all the papers on Arakawa's reaction from this Laboratory, it will be seen that α in the above expression has been centering on methyl glyoxal or a similar substance.

In 1931 Vogt-Møller stated that in animals which suffered from avitaminosis B a failure of carbohydrate metabolism would occur, that methyl glyoxal would be identified in body fluids and that beriberi symptoms were caused by methyl glyoxal intoxication. The assumption by Vogt-Møller[10] that methyl glyoxal is the toxic agent in vitamin B_1 deficiency was verified to some extent by Geiger and Rosenberg in 1933, who found it in the urines of avitaminous dogs and rats.

Whether or not methyl glyoxal is an intermediary product in normal carbohydrate metabolism is still under debate. Formerly it had been widely accepted as an essential metabolite, but since the Embden- Meyerhof

scheme was generally accepted, that idea not only has lost in favor, but been *almost entirely discarded.*

Now I have been able to isolate methyl glyoxal from human milk and identify it. It is to be noted here that the osazone of methyl glyoxal was isolable without use of monoiodoacetic acid.

1,500 cc. of human milk were used for this experiment. This material consisted of specimens from many lactants in a Well Baby Contest held in Sendai city. It was not difficult to suppose that from our experience hitherto (especially in the postbellum times) only a small percentage of these cases should have shown positive Arakawa's reaction, and a portion of this collected milk tested was actually negative to Arakawa's reaction or completely Arakawa-negative (showing still negative reaction after a lapse of 5 minutes). 10 cc. of toluene and 1 g. of monoiodoacetic acid were kept in each of 3 large flasks gathering human milk, and each flask with 500 cc. milk was kept in a refrigerator.

It was within 5 hours at the latest after gathering human milk that ether extraction of the milk was begun by Schmalfuss and Werner's extraction apparatus. Milk was poured in extraction apparatus and ether was added gently. Ether extraction was continued for about 3 hours.

The ether extract was transferred into a flask and the ether was evaporated. To this almost colorless fluid, water was added and steam distillation was performed.

The fraction was received in a receiver containing ca. 200 cc. of 0.1% 2N HCl 2,4-dinitrophenylhydrazine solution. Each drop caused yellow turbidity immediately. After a time (varying from 15 to ca. 30 minutes —at first shorter time, later longer time) another receiver with an equal amount of the same solution was exchanged for the receiver hitherto used. Then after a time a third receiver was exchanged for the second. In the fourth or fifth receiving bottle, yellow precipitates would not appear so easily as in the first ones. Still the distillation was kept going on, receivers exchanged twice or thrice more, till the distillation was completed. The yellow precipitates were collected. A part was taken and treated with 25% KOH solution: dark red color resulted. Preliminarily it was considered as 2:4-dinitrophenylhydrazone of *acetaldehyde;* eventually it turned out so, as will be decribed immediately.

Now the precipitates were washed with dil. HCl and with H_2O, and finally recrystallized from alcohol.

The yield of crystals was 0.484 g. in weight. M.P., 157°C. Mixture test: the mixture with 2:4-dinitrophenylhydrazone of synthethic acetaldehyde showed no lowering of the melting point.

$C_8H_8N_4O_4$

Theoretical: C=42.90% H=3.60% N=25.00%

Found: C=43.19% H=3.31% N=25.27%

Accordingly, the precipitates above described were the hydrazone of acetaldehyde itself.

Now the filtrates of the distillate from which the hydrazone of acetaldehyde had been obtained were left standing for two days.

Through this whole time (11 Sept.—13 Sept. 1950) I kept up a vigil and then *orange yellowish precipitates with reddish tone* came to be crystallized gradually. The whole mass was collected by filtrating and dried. Yield, 0.155 g. M.P., 285°C.

It showed deep blue violet reaction with 25% KOH solution and was considered as 2,4-dinitrophenylosazone of *methyl glyoxal* (or pyruvic aldehyde).

The crystals on the filter were washed with 2N HCl, H_2O and then with absolute alcohol. M.P. of the so treated crystals was 292°C. Furthermore, treating with alcohol was continued, lest alcohol soluble part should not remain adhering, and the osazone was recrystallized from nitrobenzene, and weighed after drying in a P_2O_5 dessicator. Yield, 50 mg. M.P. 297°C.

It was red microcrystalline compound with an orange tone. This osazone was identified by mixture test with synthetic methyl glyoxal bis-2,4-dinitrophenylhydrazone (M.P., 297°C.).

The mixture showed no lowering of melting point.

$C_{15}H_{12}N_8O_8$

Theoretical: C=41.70% H=2.80% N=25.90%
Found: C=41.98% H=2.70% N=26.07%

Accordingly the precipitates obtained were identified to be the osazone of methyl glyoxal itself.

It is to be noted that the mother liquid (subjected to steam distillation) was treated by adding 2,4-dinitrophenylhydrazine for possible identification of pyruvic acid.

It produced yellowish precipitates immediately; these were all identified as the hydrazones of acetaldehyde. Pyruvic acid or any remainder of methyl glyoxal was never identified.

TABLE I

2,4-Dinitrophenylhydrazones and *p*-Nitrophenylhydrazones, prepared from Human Milk and Synthetic Preparations

No. 1 (Human Milk)
Acetaldehyde-2,4-dinitrophenylhydrazone,
M. P., 152°,C, the first fraction.

No. 2 (Human Milk)
Acetaldehyde-2,4-dinitrophenylhydrazone,
M. P., 160°C, washed by alcohol.

No. 3 (Human Milk)
Acetaldehyde-2,4-dinitrophenylhydrazone,
M. P., 162°C, recrystallized by alcohol.
$C_8 H_8 N_4 O_4$

Theoretical	Found
C=42.90%	C=43.19%
H=3.60%	H=3.31%
N=25.00%	N=25.27%

No. 4 (Human Milk)
Methyl glyoxal-bis-2,4-dinitrophenylhydrazone,
M. P., 285°C, the first fraction.

No. 5 (Human Milk)
M. P., 292°C, washed by alcohol.

No. 6 (Human Milk)
Methyl glyoxal-bis-2,4-dinitrophenylhydrazone,
M. P., 297°C, recrystallized by nitrobenzol.
$C_{15} H_{12} O_8 N_8$

Theoretical	Found
C=41.70%	C=41.98%
H=2.80%	H=2.70%
N=25.90%	N=26.07%

No. 7 (Synthetic preparation)
Methyl glyoxal-bis-2,4-dinitrophenylhydrazone,
M. P., 297° C, prepared from acetone, oxidized by selenium oxide (by Prof. H. Tatsuta).

No. 8 (Synthetic preparation)
Methyl glyoxal-bis-2,4-dinitrophenylhydrazone,
M. P., 297° C, prepared from iso-nitrosoacetone (by Associate Prof. Y. Nakamura).
$C_{15} H_{12} N_8 O_8$

Theoretical	Found
C=41.66%	C=41.50%
H=2.81%	H=2.87%

No. 9 (Human Milk)
Acetaldehyde-para-nitrophenylhydrazone,
M. P., 120° C.

No. 10 (Human Milk)
Para-nitrophenylhydrazone,
M. P., 210° C, not pure, methyl glyoxal-mono-para-nitrophenylhydrazone.

No. 11 (Human Milk)
Methyl glyoxal-bis-para-nitrophenylhydrazone,
M. P., 277° C, not pure.

No. 12 (Synthetic preparation)
Acetaldehyde-2,4-dinitrophenylhydrazone,
M. P., 158° C.

No. 13 (Synthetic preparation)
Pyruvic acid-2,4-dinitrophenylhydrazone,
M. P., 213° C.

No. 14 (Synthetic preparation)
Acetaldehyde-para-nitrophenylhydrazone,
M. P., 128° C.

No. 15 (Synthetic preparation)
Methyl glyoxal-bis-para-nitrophenylhydrazone,
M. P., 302° C.

Discussion. The fractional separation of acetaldehyde and methyl glyoxal in ether extract was thus performed with success reminding me of Duclaux's success in the separation of propionic and butyric acids in metabolic products by use of "distillation ratio." (Cf. Bertrand and Thomas' " Manipulations de chimie biologique ")†

It seems that the formation of methyl glyoxal osazone in a mixture solution is relatively slow, so that it could be obtained, if the filtrate was to be left standing for a fairly longer time, while the formation of acetaldehyde hydrazone is completed immediately in the mixture solution of acetaldehyde and methyl glyoxal.

Pyruvic acid in the blood of vitamin B_1-deficent pigeons was identified by preparation and analysis of 2,4-dinitrophenylhydrazone by R.E. Johnson[12] in 1935, who however was not able to find any trace of methyl glyoxal.

This is not true for human milk. Quite the reverse is true. In human milk methyl glyoxal was identified, but pyruvic acid was not identified.

As above related methyl glyoxal has been identified in human milk by preparation and analysis of the 2,4-dinitrophenylosazone. And acetaldehyde in human milk has been identified by preparation and analysis of the 2,4-dinitrophenylhydrazone, whereas pyruvic acid has not been identified at all in human milk.

Summary. Methyl glyoxal and acetaldehyde were identified as 2,4-dinitrophenylhydrazone from human milk. Both were isolated and ascertained by synthetic preparations and by analysis of 2,4-dinitrophenylhydrazone. Pyruvic acid was not identified at all in human milk. (Cf. Summary for Parts II & III.)

Coclusions. Methyl glyoxal from human milk was isolated and identified by analysis and synthesis. *Pyruvic acid was never identified from human milk.* This is, I believe, the very first identification of methyl glyoxal from human milk by way of strict chemical determination. (Cf. Conclusions for Parts II & III.)

Part II

Isolation and identification of methyl glyoxal from human milk as *p*-nitrophenylhydrazone

Neuberg and Kobel[13] isolated in 1928 methyl glyoxal as methyl glyoxal *p*-nitrophenylhydrazone in alcohol fermentation process by yeast.

In our country T. Ariyama[14] in 1931 isolated and identified methyl glyoxal as *p*-nitrophenylhydrazone of the decomposition product of sugar by so-called " beriberi bacteria " (Matsumura).

Neuberg and Kobel noticed that *p*-nitrophenylosazone was not suitable for isolation of methyl glyoxal, because methyl glyoxal *p*-nitrophenylhydrazone showed, as they stated, rather unreliable results of analysis.

I also experienced that *p*-nitrophenylhydrazones of similar compounds were generally soluble in alcohol, making the separation of the osazones from one another rather difficult, so *p*-nitrophenylhydrazine is not suitable for the isolation of each osazone from the mixture. Still I tried, in order to make my result surer, to separate *p*-nitrophenylhydrazones extracted from human milk directly and to investigate all the ketoaldehyde components in it.

I. Direct method. I gathered ca. 500 cc. of human milk from many lactants and added 1 g. of monoiodoacetic acid and 10 cc. of toluene to it to prevent the progress of chemical reactions concerned. Fat was removed by centrifugation. Then deproteinisation was performed by adding 10 cc. of 10% metaphosphoric acid to each human milk of 40 cc. and filtering after centrifugation.

To this filtrate, 1.5 g. of *p*-nitrophenylhydrazine solved in 100 cc. of 30% acetic acid were added. Dark red crystals began to precipitate immediately. They stood overnight and were filtered. Yield, 0.65 g. after drying. M.P., 156–157° C.

As this M.P. was quite near that of *p*-nitrophenylhydrazine itself, it was treated with about 80 cc. alcohol in order to remove alcohol-soluble substances adehering to the precipitates and was then filtered. The precipitates were washed with diluted HCl solution to remove the unreacted hydrazine solution. The crystallised product was washed with H_2O and dried. M.P., 156°C. It is evident that this was not *p*-nitrophenylhydrazine itself.

Now I resorted to paper partition chromatography which will be described in details in Part III. This result showed that the substance was the mixture of osazones corresponding to more than two substances; one of them was acetaldehyde and another probably methyl glyoxal.

So I separated it into soluble and insoluble parts by hot toluene extraction. The insoluble part was extracted by alcohol. After drying the soluble part, it was crystallised by alcohol. M.P., 220°C. Yield, slight.

II. Indirect method—Ether extraction method. I tried to extract from another specimen of ca. 500 cc. of human milk (from many lactants) by

the same ether extraction method as I described in Part I of this treatise. After the ether extract was distilled, 50 cc. of water were added.

To this solution, 1.5 g. of *p*-nitrophenylhydrazine solved in 100 cc. of 30% acetic acid were added.

It began to precipitate orange yellowish crystals (the first precipitates) immediately, but the crystals and all were left standing overnight. Then it was filtered and dried. Yield, 0.12 g.; M.P., 157°C. After filtrating, it was furthermore left standing for two more days, during which time orange yellowish precipitates were seen to deposit on the bottom (the second precipitates). Yield, 0.10 g.; M.P., 157°C. The first and the second precipitates showed thus the same melting point of 157°C.

The combined crystals were divided into two parts, hot toulene soluble and hot toluene insoluble parts.

Recrystallised products from the toluene soluble and insoluble parts: M.P., 210°C and 225°C–235°C respectively.

As the yields in II (indirect method) as well as in I (direct method) were rather small, I combined the toluene soluble substances obtained in I and II on one side and the toluene insoluble substances obtained in I and II on the other.

(1) Toluene soluble part was dried under decreased pressure and it was recrystallised as dark yellow crystalls. Yield, 0.030 g.; M.P., 120°C.

The crystals showed red color with 25% alkaline alcoholic solution. It was considered as *p*-nitrophenylhydrazone of acetaldehyde.

The mixture test with pure synthetic acetaldehyde osazone showed no lowering of melting point.

It was thus acetaldehyde-*p*-nitrophenylhydrazone.

(2) The toluene insoluble part was recrystallised from the solvent mixture of nitrobenzene and toluene (1:2).

Yield, 0.02 g.; M.P. 277°C. Although melting point was far lower compared with that (297°C) of *p*-nitrophenylhydrazone, I gave up recrystallising it furthermore owing to too small an amount of the yield. It showed blue (rather violet) color reaction with alkaline solution.

The mixture test with pure synthetic methyl glyoxal-*p*-nitrophenylosazone showed no lowering of melting point.

It was thus considered as methyl glyoxal-*p*-nitrophenylhydrazone.

(3) In the case of drying the toluene soluble and insoluble parts above mentioned, slight yellowish microcrystals were sublimed during the course of recrystallisation. Yield, slight; M.P., 168°C sharp.

It showed no color reaction with alkaline solution. This substance remains unknown.

(4) In the case of the toluene insoluble part from recrystallisation

by 50% ethanolic solution, dark red crystals were obtained.

Yield, 0.05 g.; M.P., 210°C.

The color reaction with alkaline solution was blue.

The substance might possibly be the mixture of osazones of acetaldehyde and methyl glyoxal in a certain proportion, or it might be related to methyl glyoxal-mono-*p*-nitrophenylhydrazone (M.P., 218°C).

Although the separation of acetaldehyde and methyl glyoxal in human milk by use of *p*-nitrophenylhydrazine was very difficult, I was able to identify them at all.

Acetaldehyde-*p*-nitrophenylhydrazone was obtained from the toluene soluble part in human milk.

The methyl glyoxal-*p*-nitrophenylosazone was separated and identified by selecting suitable solvents. The melting point of the substance was lower than that of the synthetic osazone, as the yield was rather small. Repeated recrystallisation was impossible.

The *p*-nitrophenylhydrazones which had M.P. 156–157°C and 210°C respectively were also obtained.

Pyruvic acid was neither isolated nor identified.‡

Some readers might possibly wonder why I had undertaken the work described in Part II, which would involve much more difficulty. The identification of pyruvic aldehyde and acid had not been my sole purpose, but during the work described in Part II, I thought I might have come across some other ketoaldehydes in human milk.

Summary. Methyl glyoxal and acetaldehyde were identified as *p*-nitrophenylhydrazone from human milk.

Acetaldehyde was identified by synthetic preparation. The treatment of methyl glyoxal hydrazone was, as had been expected, much more difficult than in the case of 2,4-dinitrophenylhydrazone. The amount available was not large enough to make minute comparison with synthetic preparations. (Cf. Summary for Parts I & III.)

Conclusions. Methyl glyoxal from human milk was isolated and identified by analysis and synthesis. Pyruvic acid was never identified from human milk. (Cf. Conclusions for Parts I & III).

Conclusions

Methyl glyoxal (pyruvic aldehyde or pyruvaldehyde) was isolated from human milk (negative to Arakawa's reaction) for the first time in the form of 2,4-dinitrophenylhydrazones as well as *p*-nitrophenylhydrazones.

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‡ at least, not in an amount large enough to be isolated.

The Tohoku Journal of Experimental Medicine, Vol. 57, Nos. 2-3, 1953

The Isolation and Identification of Methyl Glyoxal

(Pyruvaldehyde or Pyruvic Aldehyde) from Human Milk

Part III

Identification of acetaldehyde and methyl glyoxal from human milk by paper partition chromatography

265th Report of the Peroxidase Reaction
(212th Human Milk Study)

By

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(Received for publication, July 21, 1952)

Introduction

The Introduction common to Parts I, II and III of the present treatise was related in the beginning of Part I. The Preliminary Report[1] to this treatise was already published in this Journal of the present treatise. Now the introduction to Part III alone will be stated here.

The separation of amino acids by partition chromatography on paper was first described in 1944 by Consden, Gordon and Martin.[15] A large number of separation of organic compounds have been described since their publication.

By this method, I was able to identify *methyl glyoxal in human milk*, but as to the occurrence of pyruvic acid in human milk, I was not able to prove it.

Thus I succeeded in verifying by using another method of identification—paper partition chromatography—my own results described already in Part I and Part II of the present treatise.

Experimental

A cylinder of Toyo-filter paper No. 2 for qualitative analysis, 20 cm. high and 15 cm. wide was used, and drops of test solution to be analysed were placed 5 cm. from the bottom of the cylinder and marked in pencil. The cylinder was developed by letting it stand in a dish for 1~2 hours

* Now Professor Emeritus

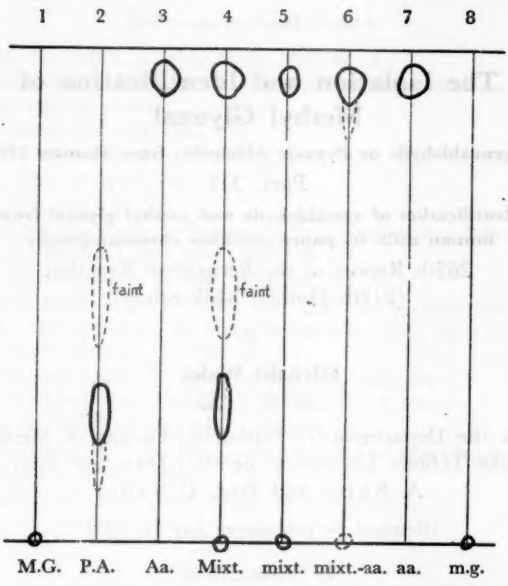

Fig. 1. Paper chromatogram of 2,4-dinitrophenylhydrazones prepared from human milk and synthetic preparations; solvent; n-butanol. color of spot; yellowish.

1. and M.G.: synthetic methyl glyoxal osazone. 2. and P.A.: synthetic pyruvic acid hydrazone. 3. and Aa.: synthetic acetaldehyde hydrazone. 4. and Mixt.: synthetic mixture of acetaldehyde, pyruvic acid and methyl glyoxal hydrazones. 5. and mixt.: mixture hydrazones prepared from human milk. 6. and mixt-aa.: mixture hydrazones (acetaldehyde hydrazones preliminarily excluded). 7. and aa.: acetaldehyde hydrazone prepared from human milk. 8. and m.g.: methyl glyoxal osazone prepared from milk.

in the case of xylene acetic acid and 5~6 hours in the case of *n*-butanol.

The mixture solution of water saturated xylene and acetic acid (4:1) was suitable for *p*-nitrophenylhydrazones and water saturated *n*-butanol is suitable for 2,4-dinitro-phenylhydrazones.

The separation takes place as is shown in accompanying illustration (Fig 1. & Fig 2.).

Synthetic methyl glyoxal (M.G.) was deposited on the point where the drop was applied (R_f=O), by 2,4-dinitrophenylhydrazone and in the case of *p*-nitrophenylhydrazone, the R_f value was 0.2.

Synthetic pyruvic acid hydrazone (P.A.) travels rather slowly (R_f= 0.29) by 2,4-dinitrophenylhydrazone and (R_f=0.22) by *p*-nitrophenylhydrazone. Synthetic acetaldehyde hydrazone (Aa.) develops easily and

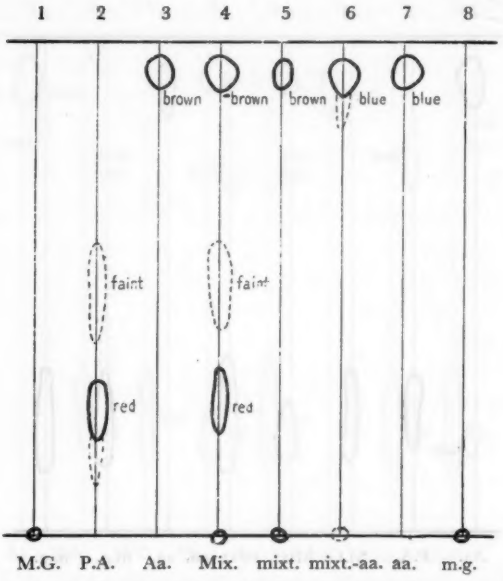

Fig. 2. Paper chromatogram of 2,4-dinitrophenylhydrazones extracted from human milk and synthetic preparations; color reactions spraying by caustic ethanolic solution. Numbers and abbreviations signify the same as in Fig. 1.

has R_f value of 0.94–0.95. (Cf. Table I).

The spots of synthetic acetaldehyde and pyruvic acid and methyl glyoxal osazones were at first yellow or orange.

By spraying 25% KOH 50% ethanolic solution, they showed brown, for acetaldehyde (Aa., Fig. 2), portwine-like red for pyruvic acid hydrazones (P.A., Fig. 2) and deep blue color for methyl glyoxal osazones (M.G., Fig. 2) respectively.

The hydrazone (mixt-aa.) prepared from human milk showed two spots for acetaldehyde and methyl glyoxal as 2,4-dinitrophenylosazone as well as *p*-nitrophenylosazone.

The spots for pyruvic acid from human milk was not identified. The method here described has, as is well known, shown the great advantage of separating different substances of minute amounts extracted and prepared from different materials. The material was human milk in my case.

Discussion

The hydrazones of synthetic acetaldehyde, synthetic pyruvic acid

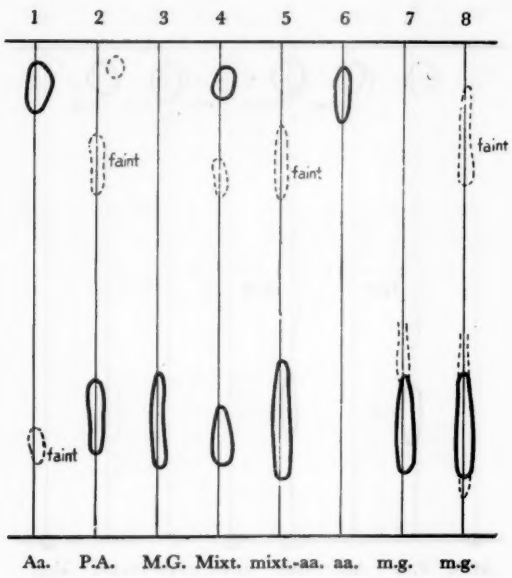

Fig. 3. Paper chromatogram of *p*-nitrophenylhydrazones prepared from human milk and synthetic preparations; solvent; xylene: acetic acid (4:1). Color of spot; yellowish.

1. and Aa: synthetic acetaldehyde hydrazone. 2. and P.A.: synthetic pyruvic acid hydrazone. 3. and M.G.: synthetic methyl glyoxal osazone. 4. and Mixt.: synthetic mixture hydrazones of acetaldehyde, pyruvic acid, and methyl glyoxal. 5. and mixt-aa.: mixture hydrazones prepared from human milk, (acetaldehyde hydrazone preliminarily excluded). 6. and a.a.: acetaldehyde hydrazone prepared from human milk. 7. and m.g.: methyl glyoxal osazone prepared from human milk. M. P., 277° C. 8. and m.g.: methyl glyoxal osazone prepared from human milk. M. P., 210° C., not recrystallised, not pure.

and synthetic methyl glyoxal showed their proper R_f value.

It is to be noted here that, as is shown in Fig 2, in spite of my endeavor to purify the osazone, synthetic pyruvic acid has showed, besides its proper R_f value (R_f=0.29), the other R_f value (R_f=0.5), (P.A., the upper spot and Mixt., the upper middle spot in Fig. 1) and that methyl glyoxal crystallised from milk extract showed, besides its proper R_f value (R_f=O), the other R_f value (mixt. -aa, Fig. 2) though its color reaction was similar to that of the proper spot (R_f=O) of methyl glyoxal.

The cause for such a phenomenon may be: hydrazones would form ones of different proportions which might show sometimes a different R_f value other than that of proper hydrazones which would generally

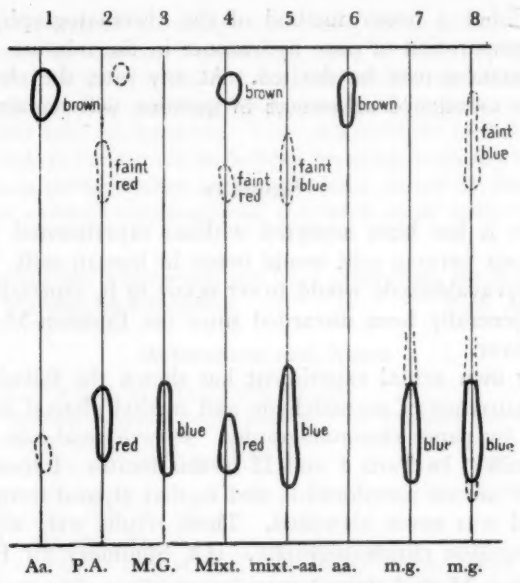

Fig. 4. Paper chromatogram of *p*-nitrophenylhydrazones prepared from human milk and synthetic preparation; color reactions spraying by caustic ethanolic solution. Numbers and abbreviations signify the same as in Fig. 3.

TABLE I

R_f value and Color Reaction in Paper Chromatogram of Hydrazones, extracted from Human Milk and Synthetic

Substance	R_f value of 2, 4-dinitrophenylhydrazone		R_f value of *p*-nitrophenylhydrazone		Color reaction of alkali ethanolic solution
	Extracted from human milk	Synthetic	Extracted from human milk	Synthetic	
Acetaldehyde	a) 0.95	a) 0.94–0.95	a) 0.95	a) 0.94–0.95 b) 0.20	Brownish (dark red)
Pyruvic acid	a) (*) b) (*)	a) 0.29 b) 0.50	a) (*) b) (*) c) (*)	a) 0.22 b) 0.80 c) 0.94–0.95	Red, portwine-like
Methyl glyoxal	a) =0	a) 0 b) 0.94	a) 0.20	a) 0.20 b) 0.79	Blue

a)*, b)*, c)*, Pyruvic acid was never identified.

be expected.

There are a couple of possibilities at least for the solution of such a

problem. Either a better method of the chromatography or a better method of preparation of pure hydrazones in the mixture of hydrazones forming substances may be devised. At any rate, the chemical nature of the above mentioned substances in question will remain to be solved in future.[16]

Summary

Hitherto it has been accepted without experimental evidence that in athiaminosis pyruvic acid would occur in human milk, while methyl glyoxal or pyruvaldehyde would never occur in it, especially as this substance has generally been discarded since the Embden-Meyerhof theory came into favor.

But my own actual experiment has shown the following results:

The occurrence of acetaldehyde and methyl glyoxal in human milk was assured by paper chromatography. Pyruvic acid was not identified in human milk. In Parts I and II of this treatise, I stated that I had isolated with success acetaldehyde and methyl glyoxal from human milk. Pyruvic acid was never identified. These results were also ascertained by paper partition chromatography. (Cf. Summary for Parts I & II.)

Remarks. Methyl glyoxal as an intermediate of normal carbohydrate metabolism has, as has been stated in Introduction of Part I, lost in favor. Not only that, but also it has been considered sometimes as secondarily produced or even an " artifact " due to the instability of such substances as glyceraldehyde, dioxyacetone, diacetyl, acetol or lactic aldehyde, from which (esp. the first) the glyoxal might be produced. But now I firmly believe, on the basis of many Reports on Human Milk Study from our Laboratory, that there is an abnormal and quite a natural course of production of methyl glyoxal which, in normal metabolism, will not occur in an identifiable form or, if at all, occur in a trace.

Conclusions

The results obtained in Parts I, II and III of the present treatise will lead to the following conclusions.

Methyl glyoxal was isolated from human milk. It was identified analytically as well as by paper partition chromatography.

Acetaldehyde was also identified in human milk.

Pyruvic acid was neither isolated nor identified from human milk; it was not identified by paper partition chromatography either. (Cf. Conclusions for Parts I & II.)

I am very grateful to Associate Prof. N. Nakamura (Biochemisty) of our University, who is especially interested in carbonyl groups himself, for furnishing

me with much practical knowledge concerning the problem, and to Prof. H. Tatsuta, Professor of Organic Chemistry in our University, for giving me every assistance needed for the synthetic preparations and the identification of methyl glyoxal. I desire to avail myself of this opportunity to express my hearty thanks to them for their kind co-operation. I am also indebted to Prof. T. Sano who was kind enough to furnish me with those materials necessary for the investigation, who was perhaps more enthusiastic than myself in this problem and without whose constant encouragment this work could never have been accomplished.

This investigation was carried out by a grant from the Science Research Fund of the Ministry of Education. Prof. Akira Sato.

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16) The solvent used for these substances may have been inappropriate, concerning which I expect to be able to report in a coming issue of this Journal.

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Contribution to Pathogenesis of Transient Infantile Hemiplegia probably due to Izumi Fever; with Special Reference to "The Cross Section Pneumoencephalograms"

By

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(Received for publication, July 21, 1952)

Strümpell's hemiplegia or acquired infantile hemiplegia is a rare disease in children and the etiology of this illness was discussed in detail by Ford and Schaffer.[1] According to them[1] clinical pictures of this disease are as follows: the onset is abrupt and attended by fever, convulsions and disturbance of consciousness, and spastic hemiplegia is noted within a few hours or a day after the onset; in the next few days the child improves rapidly, but the hemiplegia is usually, not invariably, permanent. This disease may develop in apparently healthy children or may be associated with acute infectious diseases.

Ford and Schaffer[1] stated that the infantile hemiplegia that was associated with acute infectious diseases was probably due to vascular lesions and that the hemiplegia which occurred only in the course of these illnesses was, in the majority of cases, due to invasion of the blood stream by the primary organism or by some secondary invader, and that the palsies that developed during convalescence were the result of the vascular lesions in the cerebral arteries.

Concerning the cause of the hemiplegia that occurs in apparently healthy children, Ford and Schaffer[1] reported that: (1) the obvious assumption of encephalitis was not supported by anatomical evidence; (2) only vascular lesions had been studied, and these vascular lesions might have been related to the previous illness that these children had suffered, and thus it was possible that invasion to blood stream by pyogenic organisms from clinically latent foci of infection might have been the cause of some instance of this nature.

These criteria respecting the etiology of this illness were made subject to correction in terms of histopathological findings of the brains,

and Ford and Schaffer[1] stated that nothing was known of the cause of the transient hemiplegia.

In the present paper we shall report a case of Strümpell's hemiplegia with complete recovery from hemiplegia, and we will discuss the etiology of this illness from the view point of the findings of "the cross section pneumoencephalograms."[2),3),4)]

Report of Case

Clinical history:—K.K., a boy, aged 11 years, brought into our Clinic in a state of coma, was the child of healthy parents. Birth had been normal and there was no evidence of birth injury. Growth and development had been normal in every respect. His general health had always been good and there was no record of any illness. The day before he was brought to us (at about 7.00 a.m. on 12 Nov. 1951), severe general convulsions occurred suddenly, and the child remained unconscious for about an hour after the seizures. After the recovery from unconsciousness he complained of severe headache and his temperature had risen to 38.5°. He was brought to our Clinic 30 hours after the onset.

Physical examination:—The patient was a well-developed, fairly well nourished boy, whose temperature was 40°. He was stuporous. His face was flushed and there was a distinct facial palsy on the left side. The throat, chest and abdomen showed no abnormalities. The skin was without eruption. The left arm and left leg were apparently paralyzed. Rigidity of the arms and legs was found, on passive movement on both sides, but it was found to be more distinct on the left side. Knee jerks were moderately exaggerated on both sides, especially on the left. Babinski's, Oppenheim's and Goldon's reflexes were elicited on both sides, more distinctly on the left. Ankle-clonus was negative on both sides. Abdominal reflex was absent on the left side.

Course and laboratory findings:—His temperature fluctuated from 37.5° to 39° for only 8 days after the onset of this illness. No attacks of convulsion developed after the second day. Disturbance of consciousness, headache, incontenentia alvi and urinae and facial palsy were improved rapidly by the 6th day. The left-sided hemiplegia was improved slightly on the 6th day and had almost completely disappeared by the 14th day. Exaggeration of knee jerks and rigidity of the arms and legs disappeared simultaneously with the recovery from hemiplegia) It is worth while to mention that membranous desquamation was observed on the fingers and toes after the 11th day of the illness.

Blood pictures:—on the 3rd day, red corpuscles 3.83 millions, hemoglobin 10.5 g. per 100 cc., M.C.V. 112 μ^3, white cells 8,600, eosinophils 0% (0) lymphocytes 11% (940), monocytes 3% 258), neutrophils 86% (7,396), and nuclear shift of neutrophils-I (3%) II (37%) III (49%), IV (11%) and V (0%); on the 19th day, red corpuscles 4.10 millions, hemoglobin 11.5 g per 100 cc., M.C.V. 107μ^3, white cells 6,400, eosinophils 5% (320), lymphocytes 50% (3,200), monocytes 3% (192), neutrophils 50% (3,500), and nuclear shift of neutrophils-I (13%) II (34%) III (38%) IV (12%) and V (3%).

Cerebrospinal fluid—As will be seen from Table I, the pressure was increased but not very high even in the early stage of the illness. Nonne's reaction remained negative throughout the course of the disease, while Pandy's reaction, which had been negative by the 3rd day, became positive on the 6th day and remained positive to the 14th day. Sugar content in the fluid was increased and ranged from 0.1% to 0.3%, and glycosuria was found on the 6th day. Cells in the fluid were mononuclear, and cell count, which had been within the normal range by the second day, was increased markedly on about the 6th day. No known microorganisms were found on the culture of the spinal fluid taken on the 6th day.

TABLE I

Findings of the Cerebrospinal Fluid

Date	13/XI	14/XI	17/XI	19/XI	25/XI	28/XI
Day of illness	2	3	6	8	14	17
Pressure mmH_2O	200	250	150	150	150	150
Nonne's reaction	(−)	(−)	(−)	(−)	(−)	(−)
Pandy's reaction	(−)	(−)	(+)	(+)	(+)	(−)
Sugar (%)	0.1	0.3	0.2	0.2	0.1	0.1
Cell count	6/3	38/3	132/3	108/3	40/3	23/3

"*Cross section pneumoencephalograms*":—Pneumoencephalograms were taken after introducing air in an amount of 170 cc. after tapping out 120 cc. of the spinal fluid in the sitting position.

In the pneumoencephalograms taken on the third day of the illness, the third ventricle and lateral ventricles were not demonstrated.

The pneumoencephalograms and "cross section radiograms of ventricles"[2),3),4)] were taken again on the 9th day (Cf. Figs. 1, 2, 3 and 4).

Fig. 1

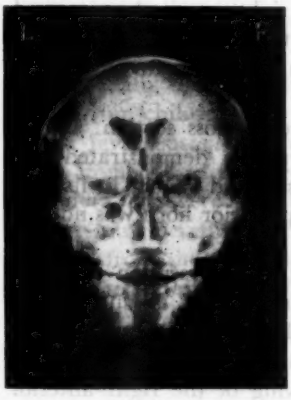

Fig. 2

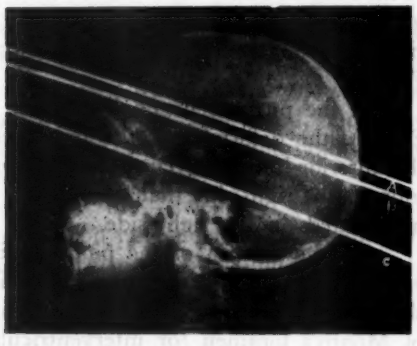

Fig. 1. Pneumoencephalogram taken on the 9th day.

Fig. 2. Pneumoencephalogram taken on the 9th day. Lines A, B, and C demonstrate the levels of "the first cross section," "the second cross section" and "the base cross section" respectively.

The pneumoencephalogram of "the first cross section",[2)] which will reveal a cross section figure of the brain at the level of line-A in Fig. 2, showed the shadows of the anterior horns on both sides, the third ventricle and the left posterior horn, but not that of the right posterior horn (Cf.

Fig. 3

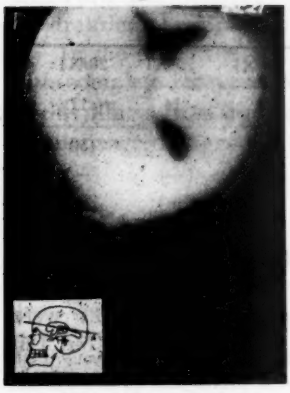

Fig. 4

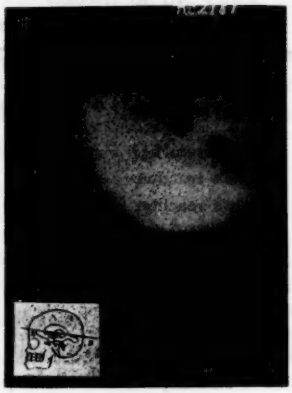

Fig. 3. "The cross section pneumoencephalogram" taken on the 9th day. The attached schema demonstrates the level of "the first cross section."

Fig. 4. "The cross section pneumoencephalogram" taken on the 9th day. The attached schema demonstrates the level of "the second cross section."

Fig. 3). In Fig. 3, the right anterior horn was smaller in size than that of the left; in other words the lateral wall of the right anterior horn seemed to be situated, with a slight convexity, nearer to its median wall. The right wall of the third ventricle showed a slight convexity to the left in Fig. 3.

In the pneumoencephalogram of "the second cross section"[2] (Cf. Fig. 4), the deformity of the third ventricle was not demonstrated and the anterior horn was revealed to be smaller in size and vaguer in its contour on the right side than on the left, and the posterior horn was not demonstrated on the right side.

On the criteria of the pictures given by the cross section pneumoencephalograms, it may be worth while to mention that absence of the shadows of ventricles does not always indicate the existence of obstruction of Monro's foramen (or interventricular foramens).[3]

We shall, therefore, emphasize the narrowing of the right anterior horn due to 'abnormal' convexity of its lateral wall as well as the 'abnormal' convexity of the right wall of the third ventricle; these findings may be explained by a "swelling" (an increase in volume) of the brain mass which forms the lateral wall of the right anterior horn and the right wall of the third ventricle.

Comment

According to Ford and Schaffer[1]: (a) permanent hemiplegia which

occurred in acquired infantile hemiplegia was due to vascular lesions which weakened the cerebral arteries and tended to cause cerebral hemorrhage; (b) nothing is known concerning the etiology of the transient hemiplegia which occurred in this illness.

In our case of acquired infantile hemiplegia, the hemiplegia lasted for only 10 days after the onset of the illness and disappeared completely.

From the cross section pneumoencephalograms of our case it may be suggested that "swelling" of the brain existed on both sides (at least by the third day of the disease) and that "swelling" was more intense on the right side.

Also the results of the examination of the spinal fluid gave no evidence against the existence of cerebral "swelling."

The probable existence of the "swelling" of the brain, which was probably more severe on the right side, was probably a factor contributing to the pathogenesis of the transient hemiplegia.

What could have been the cause of the "swelling" of the brain? The child showed membranous desquamation on the fingers and toes after the 10th day of illness. This finding made us suggest possible infection by Izumi fever[5),6),7)], because sporadic occurrence of Izumi fever was observed around the district where the patient has lived, and membranous desquamation on the fingers and toes not uncommonly is a sole clue for the diagnosis of Izumi fever.[7)] As already reported by Prof. Izumi,[5)] cerebral symptoms are rarely associated with Izumi fever.

K. Sato, Professor of Pathology at Hirosaki University, obtained blood from children who had been diagnosed by Arakawa (one of us), as Izumi fever cases, innoculated guinea pigs with this blood, autopsied them from five to ten days later, and found both hyperemia and edema, considerably severe in intensity, in the brain tissues.

It may be, therefore, concluded that the "swelling" of the brain inferred from the results of the cross section pneumoencephalograms may be edema and/or hyperemia of the brain, caused by Izumi fever.

Conclusions

In a case of acquired infantile hemiplegia, "the cross section pneumoencephalograms" were studied, and the transient hemiplegia was ascribed to a sudden onset of cerebral edema which was probably more severe on the right side and which was probably cuased by Izumi fever.

We wish to thank Dr. Sh. Takahashi, Professor of Radiology, for taking the cross section pneumoencephalograms.

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The Tohoku Journal of Experimental Medicine, Vol. 57, Nos. 2-3, 1953

Electrophoretic Analysis of Serum Protein of Children with or without Nutritional Dystrophy

Studies on the Nutrition of Children in the Hirosaki Area

(14th Report)

By

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(Received for publication July 21, 1952)

As a part of our investigations[1)2)3)] on nutritional dystrophy of children in the Hirosaki Area, electrophoretic patterns of sera from children with or without nutritional dystrophy are reported.

Method and Materials

Electrophoretic analysis of serum protein was carried out by means of Tiselius' apparatus (Type HT-B, Hitachi Seisakusho, Japan), according to Hirai's description.[4)5)] Total protein of the sera was estimated refractometrically by means of a hand-refractometer[6)] (Hitachi Seisakusho, Japan).

The children subjected to this investigation were divided into three groups as follows: Group I included children who were healthy and attending primary school in Hirosaki City and were free from any evidence of nutritional dystrophy; Group II included those who were apparently healthy and attending primary school in Watoku village, near Hirosaki City and had no complaints of malaise, but showed, on close examination, one or more of the clinical manifestations of nutritional dystrophy such as angular stomatitis, hyperpigmentation of the skin and lingual changes; Group III included those who clearly showed the symptoms of nutritional dystrophy and/or were suffering from disease or diseases other than nutritional dystrophy.

Results and Comment

The results obtained are given in Tables I, II and III.

Total protein:—Total protein was found in the averages of 6.76± 0.19, 6.83±6.09 and 6.62±0.63 g. per 100 cc. for Groups I, II and III re-

TABLE I

Electrophoretic Components of the Sera from the Children belonging to Group I

No. of case	Name, sex and age (y.)	Total protein (g. per 100cc.)	Albumin (%)	Globulin (%)			A/G
				α	β	γ	
1	I.K. ♂ 7.	6.82	55	9	13	23	1.22
2	I.S. ♂ 7.	6.72	66	9	12	13	1.94
3	S.M. ♂ 7.	6.42	53	9	16	22	1.13
4	A.S. ♂ 7.	6.62	59	12	14	15	1.44
5	K.S. ♂ 8.	6.74	54	11	13	22	1.17
6	H.K. ♂ 8.	6.64	55	7	12	26	1.22
7	K.K. ♂ 8.	6.54	59	10	13	18	1.44
8	S.U. ♂ 8.	6.44	64	8	11	17	1.78
9	T.K. ♂ 9.	7.07	60	11	12	17	1.50
10	T.K. ♂ 9.	6.57	59	11	13	17	1.49
11	B.T. ♂ 9.	7.17	51	10	12	26	1.08
12	Y.O. ♂ 9.	6.47	59	8	11	22	1.44
13	O.N. ♂ 10.	6.44	68	11	11	10	2.13
14	S.T. ♂ 10.	6.24	66	10	11	13	1.94
15	S.F. ♂ 10.	7.24	59	10	14	17	1.44
16	K.N. ♂ 11.	7.38					
17	T.K. ♂ 11.	6.68	58	13	12	17	1.39
18	K.T. ♂ 11.	6.68	63	9	11	17	1.70
19	N.T. ♂ 11.	7.18					
20	S.M. ♂ 11.	6.98	55	13	11	21	1.22
21	O.C. ♂ 11.	6.98	52	11	16	21	1.08
Average		6.76 ±0.19	58.2 ± 2.5	10.1 ± 0.8	12.5 ± 0.8	18.6 ± 1.9	1.46 ±0.14

spectively; there were no significant differences among these three values.

Albumin:—Albumin was found in the averages of 68.7±2.5, 55.3±2.1 and 44.0±1.8% for Groups I, II and III respectively; the average of albumin for Group III was, with significant difference, the lowest.

α-Globulin:—The averages of α-globulin were 10.1±0.8, 9.7±0.9 and 15.3+1.8% for Groups I, II and III respectively; the average of α-globulin for Group III was the highest, but there was no significant difference between the averages of Groups I and II.

β-Globulin:—The averages of β-globulin were 12.5±0.8, 12.7±0.8 and 15.3±1.8% for Groups I, II and III respectively; there were no signigicant differences among these three values.

TABLE II

Electrophoretic Components of the Sera from the Children belonging to Group II

No. of case	Name, sex and age (y.)	Total protein (g. per 100cc.)	Albumih (%)	Globulin (%)			A/G
				α	β	γ	
1	O.E. ♂ 11.	6.76	56	11	13	20	1.27
2	K.T. ♂ 11.	6.16	60	9	12	19	1.50
3	A.M. ♂ 10.	6.86	51	9	14	24	1.08
4	S.N. ♂ 10.	6.56	59	11	12	18	1.44
5	A.N. ♂ 10.	7.36	55	10	14	21	1.22
6	O.T. ♂ 9.	7.06					
7	S.M. ♂ 9.	6.77	60	6	9	25	1.50
8	K.J. ♂ 9.	6.67	52	12	16	20	1.08
9	S.N. ♂ 9.	6.57	57	6	12	25	1.31
10	T.M. ♂ 9.	6.47	57	6	16	21	1.33
11	O.S. ♂ 9.	7.21	55	12	13	20	1.22
12	S.T. ♂ 9.	7.11	55	11	13	21	1.22
13	S.Y. ♂ 9.	6.66	56	8	12	24	1.27
14	A.K. ♂ 9.	7.06	55	8	13	24	1.22
15	O.T. ♂ 7.	6.76	60	6	12	22	1.50
16	F.S. ♂ 7.	7.36	66	9	13	12	1.94
17	S.C. ♂ 7.	6.51	47	11	13	29	0.89
18	N.S. ♂ 7.	6.71	58	8	11	23	1.38
19	O.T. ♂ 7.	6.61	47	13	12	28	0.89
20	K.A. ♂ 7.	7.11	51	13	13	23	1.04
21	A.A. ♂ 8.	6.72	48	12	13	27	0.92
22	M.Y. ♂ 8.	7.32	54	12	10	24	1.17
23	A.T. ♂ 8.	7.02	50	11	15	24	1.00
24	O.T. ♂ 8.	6.42	63	9	12	16	1.70
Average		6.83 ±0.09	55.3 ± 2.1	9.7 ±0·9	12.7 ± 0.8	22.2 ± 1.5	1.26 ±0.13

γ-Globulin:—The averages of γ-globulin were 18.6±1.9, 22.2±1.5 and 25.0±2.9% for Groups I, II and III respectively. The averages of both Groups II and III were higher than the average of Group I.

Albumin/Globulin ratio:—A/G ratios were 1.46±0.14, 1.26±0.13 and 0.84±0.11 for Groups I, II and III rdspectively; A/G was the highest in Group I, middle in Group II and the lowest in Group III.

It may be concluded that; (1) respecting total protein and β-globulin, there were no significant differences among Groups I, II and III; (2) a decrease in albumin accompanied by an increase in γ-globulin was

TABLE III

Electrophoretic Components of the Sera from the Children belonging to Group III

No. of case	Name, sex and age (y.m.)	Clinical diagnosis	Total protein (g. per 100cc.)	Albumin (%)	Globulin (%)			A/G
					α	β	γ	
1	K.A. ♀ 4.7.	N.D.*	6.83	50.0	10.9	17.5	21.6	1.00
2	S.K. ♂ 7.6.	N.D.*+pertussis	7.13	40.7	18.4	16.5	24.4	0.69
3	S.T. ♀ 1.8.	N.D.*+bronchopneumonia	5.28	30.4	12.4	26.3	30.9	0.44
4	Y.A. ♂ 0.3.	N.D.*+bronchopneumonia	3.72	46.4	14.5	10.1	29.0	0.87
5	S.K. ♂ 1.5.	N.D.*+pertussis	7.46	45.6	12.3	13.2	28.9	0.84
6	I.K. ♀ 5.2.	N.D.*+lymphadenitis simplex	7.30	48.5	12.5	12.5	26.5	0.94
7	F.Y. ♂ 6.3.	N.D.*+diarrhea	6.61	50.3	12.8	11.9	25.0	1.01
8	J.T. ♀ 3.11.	N.D.*+pharyngobrochitis	6.43	45.1	16.1	13.1	25.7	0.82
9	F.Y. ♂ 6.3.	N.D.*	6.54	52.1	19.1	16.0	12.8	1.09
10	K.M. ♂ 4.9.	N.D.*	6.73	42.5	20.2	16.4	20.9	0.74
11	O.M. ♂ 7.2.	N.D.*	6.61	46.2	13.9	19.1	20.8	0.86
12	S.M. ♂ 0.10.	N.D.*+rickets	7.08	41.1	16.3	16.7	25.9	0.70
13	I.T. ♂ 5.0.	N.D.*+otitis media	8.43	33.5	19.6	14.1	32.8	1.02
Average			6.62 ±0.63	44.0 ± 1.8	15.3 ±1.8	15.6 ±2.4	25.0 ±2.9	0.84 ±0.11

* N.D.=Nutritional Dystrophy

characteristic for the electrophoresis in Group III; and (3) an increase in γ-globulin as well as a tendency toward a decrease in albumin was observed in the electrophoretic pattern of Group II as compared with that of Group I.

'Normal value' for each component of sera of Japanese children obtained by electrophoretic analysis is not established at the present time. According to Fukushima *et al.*,[7] 'normal values' for electrophoretic components in Japanese adults were given as follows: albumin was 63.7 (66.5–59.7)%, α-globulin 6.4 (8.8–4.1) %, β-globulin 9.9 (12.2-8.8) % and γ-globulin 20.4 (23.0–16.3)%.

According to Lubschez[8] the mean values given by electrophoresis of sera from normal children were about the same as those from adults, although the ranges were considerably greater.

The values for electrophoretic components of sera from the children belonging to Group I corresponded fairly closely to those obtained from 'normal' adults by Fukushima *et al.*,[7] so the electrophoretic pattern of

Group I may be taken as that of 'normal' children in Japan.

In investigations upon malignant malnutrition among children in South Africa Anderson and Altmann[9] found that its electrophoretic pattern was characterized by an increase in γ-globulin accompanied by a decrease in albumin, and they stated that there is no difference between the values found for nutritional edema and those with the additional signs of the syndrome of malignant malnutrition, and that this similarity afforded support for the opinion that malignant malnutrition is primarily a result of protein deficiency and that the manifestations of vitamin dificiency are only incidental.

Wada and Sato[3] in our Laboratory have investigated the relation between macrocytic anemia and liver biopsy findings in children with or without nutritional dystrophy, and fatty infiltration or portal fibrosis associated with diffuse fibrosis of the liver was demonstrated by them in a considerable number of cases of the biopsied specimens obtained from children with nutritional dystrophy. These histopathological changes of the liver, according to Wada and Sato, may be ascribed to a deficiency in animal protein in diet.

The relation between histopathological changes in the biopsied liver and the electrophoretic pattern of sera from the children with or without nutritional dystrophy, will be reported in detail in this Journal in the near future.

Summary

Electrophoretic analysis of sera was carried out on 21 cases of 'normal' children (Group I), 24 cases of children who were apparently healthy, but showed clinical manifestation of nutritional dystrophy to some extent (Group II), and 13 cases of sick children who showed distinct symptoms of nutritional dystrophy (Group III).

For Group I, total protein, albumin, α-globulin, β-globulin and γ-globulin were found in the averages of 6.76 ± 0.19 g. per 100 cc., $58.7\pm2.5\%$, $10.1\pm0.8\%$, $12.5\pm0.8\%$ and $18.6\pm1.9\%$ respectively; for Group II, 6.83 ± 0.09 g. per 100 cc., 55.3 ± 2.1 %, $9.7\pm0.9\%$, $12.7\pm0.8\%$ and $22.2\pm1.5\%$ respectively; and for Group III, 6.62 ± 0.63 g. per 100 cc., $44.0\pm1.8\%$, $15.3\pm1.8\%$, $15.6\pm2.4\%$ and $25.0\pm2.9\%$ respectively.

Conclusions

Abnormality in the electrophoretic pattern, characterized by a decrease in albumin accompanied by an increase in alpha and gamma globulin, was found in sick children with clear symptoms of nutritional dystrophy.

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Electrophoresis of Sera, Liver Biopsy Finding and Sato's Bromsulfalein Test in Children with Negative Response to the Ordinary Bromsulfalein Test

Studies on the Nutrition of Children in the Hirosaki Area

(15th Report)

By

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Introduction

Nutritional dystrophy with macrocytic anemia is not uncommonly found among children in the Hirosaki Area.[1] Wada and Sato,[2] one of the authors of this article, have carried out an investigation upon macrocytic anemia and its relation to liver biopsy findings in children with nutritional dystrophy, and they found that there was a certain relation between macrocytic anemias (Types I and II) and histopathological changes in the biopsied liver and that macrocytic anemia of Type I (an anemia with an increased volume index) was frequently found in cases with histopathological change of "cloudy swelling" in the biopsied liver.

Sato[3] subsequently demonstrated that "cloudy swelling" of liver cells revealed by needle biopsy, in general, could not be disclosed by means of the ordinary bromsulfalein test using the dye in an amount of 5 mg. per kg. of body weight, but that an existence of "cloudy swelling" was frequently suggested when a positive response was obtained, by means of Sato's modification of the bromsulfalein test, in which the dye was injected intravenously in an amount of 10 mg. per kg. of body weight.

In 1951, Ricketts[4] reported a correlation of liver biopsy findings with "liver tests," including the bromsulfalein test using the dye in an amount of 5 mg. per kg. of body weight, and he concluded that "liver tests" within normal range were not synonymous with the absence of liver pathology. Ricketts *et al.*[5] investigated the diagnostic value of liver

biopsy and electrophoretic analysis of serum protein in asymptomatic portal cirrhosis and demonstrated that slightly abnormal patterns with an increase of gamma and beta globulins were observed in some instances out of 9 cases subjected to electrophoretic analysis.

In the present paper, our investigation concerning the correlation among electrophoretic patterns, Sato's bromsulfalein test and liver biopsy findings with special reference to "cloudy swelling" of liver cells, was carried out only upon the children who visited our Clinic as patients and from whom were excluded those with a positive response to the ordinary bromsulfalein test using 5 mg. of the dye per kg. of body weight.

Experimental

Method and Material

Liver biopsy, electrophoretic analysis of sera and Sato's bromsulfalein test were performed in the same way as reported in the preceding papers.[2)3)6)]

As the subjects of this investigation, only those children who were negative to the ordinary bromsulfalein test (using the dye in an amount of 5 mg. per kg. of body weight) were chosen from the patients who visited our Clinic.

Results and Comment

The results obtained are given in Tables I (A, B,) and II (A, B).

According to the responses to Sato's bromsulfalein test and according to histopathological changes in the biopsied livers, all cases examined were divided into five groups as follows (Cf. Table III): Group A included the cases which were negative to Sato's test and showed no histopathological changes in the liver; Group B included those which were negative to Sato's test but showed histopathological changes in cytoplasm; Group C included those which were positive to Sato's test but showed no histopathological changes; Group D included those which were positive to Sato's test and showed histopathological changes in cytoplasm; and Group E included those which were positive to Sato's test and showed histopathological changes in both cytoplasm and interstitium of the livers.

(I) Relation between Sato's bromsulfalein test and histopathological changes in the biopsied livers:—As will be seen from Table III, histopathological changes in the liver were found in 5 out of 9 cases which were negative to Sato's test, and in 20 out of 26 cases which were positive to Sato's test. The incidence of histopathological changes in the biopsied liver, therefore, seems to be higher in the cases with positive response to

TABLE I (A)

Clinical Diagnosis, Total Protein and Electrophoresis of the Sera from Children with Positive Response to Sato's Bromsulfalein Test

No. of case	Name, sex and age (y.m.)	Clinical diagnosis	Total Protein (g. per 100cc.)	Electrophoretic components			
				Albumin (%)	Globulin (%)		
					α	β	γ
1	Y.Y. ♂ 0.7.	Rickets	5.38	33.9	22.5	19.9	23.7
2	M.E. ♀ 5.11.	Scarlatina	6.91	45.5	18.4	13.5	22.6
3	O.S. ♀ 1.7.	Acute pharyngitis	6.51	42.5	17.7	15.3	24.5
4	S.K. ♂ 1.5.	Pertussis+N.D.*	7.46	45.6	12.3	13.2	28.9
5	K.T. ♂ 6.1.	Ascariasis+N.D.*	7.65	30.0	8.9	14.0	47.1
6	K.A. ♀ 4.7.	N.D.*	6.83	50.0	10.9	17.5	21.6
7	J.T. ♀ 3.1.	Convalescence of Izumi fever+N.D.*	6.78	44.1	17.9	17.5	20.5
8	S.M. ♂ 7.10.	Izumi fever (the 10th day)	6.31	38.2	18.7	17.7	27.6
9	O.C. ♀ 2.8.	Tuberculous peritonitis	8.33	37.3	14.5	14.5	31.7
10	S.T. ♀ 6.10.	Tuberculous pleuro-peritonitis	5.20	33.7	15.8	15.1	35.4
11	M.K. ♂ 3.4.	Tuberculosis of the lungs	8.51	45.8	9.4	16.6	28.2
12	M.M. ♂ 7.0.	Ascariasis+Izumi fever (the 9th day)	7.42	34.8	22.7	14.8	27.7
13	F.K. ♀ 7.1.	Pleurisy with effusion	8.27	49.1	12.8	14.1	24.0
14	S.K. ♂ 2.1.	Pertussis pneumonia+N.D.*	7.13	40.7	18.4	16.5	24.4
15	M.Y. ♀ 5.2.	Ascariasis	7.53	50.4	13.4	13.0	23.2
16	K.H. ♂ 6.0.	Ascariasis	6.81	52.9	12.2	15.5	19.4
17	I.Y. ♀ 2.2.	Acute angina	7.53	58.9	13.4	11.7	16.0
18	K.I. ♀ 1.0.	Heine-Medin's disease (the 3rd day)	6.43	51.2	18.2	15.1	15.1
19	S.K. ♂ 1.7.	Bronchopneumonia+N.D.*	5.91	52.2	15.3	12.6	19.9
20	Y.M. ♂ 7.3.	Tuberculosis of hilar lymphnodes	6.51	53.6	13.7	12.9	19.8
21	Y.N. ♂ 3.1.	Hepatomegaly of unknown etiology	7.21	53.2	14.1	15.2	17.5
22	K.M. ♀ 1.11.	Epidemic vomiting	7.11	55.7	10.0	15.5	18.8
23	O.K. ♂ 0.9.	Atrophy (starch injury +rickets)	5.73	58.4	15.8	12.4	13.4
24	S.H. ♀ 1.7.	Rickets+starch injury	6.23	46.8	18.3	17.0	17.9
25	S.K. ♂ 4.3.	Ascariasis	7.01	41.9	17.0	16.8	19.3
26	M.J. ♂ 0.6.	Rickets	7.43	38.4	17.9	16.5	27.2

* N.D.=Nutritional dystrophy

TABLE I (B)

Histopathological Changes in the Biopsied Livers of of Children with Positive Reaction to Sato's Bromsulfalein Test

No.* of case	Infiltration of cells		Cnanges in cytoplasm				Fibrosis	
	Lobulus	Portal tracts	Swelling	Granular appearance	Fatty granules	Fatty drop	Diffuse	Portal
1	−	−	−	−	−	−	−	−
2	−	−	+	−	−	−	−	−
3	−	+	+	−	−	−	−	−
4	−	+	−	−	−	−	−	−
5	−	−	−	−	−	−	−	+
6	−	−	−	−	+	−	−	−
7	−	−	+	−	−	−	−	−
8	−	−	+	−	−	−	−	−
9	−	−	+	−	−	−	−	+
10	−	−	−	−	−	+	−	+
11	−	+	−	−	+	−	−	+
12	−	−	+	−	+	−	−	+
13	−	−	+	−	−	−	−	−
14	−	−	+	−	−	−	−	+
15	−	−	−	−	−	−	−	−
16	−	+	−	−	−	−	−	−
17	−	−	−	−	+	−	−	−
18	−	−	−	−	−	−	−	−
19	−	−	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−
21	−	−	−	−	+	−	−	−
22	−	−	−	−	+	−	−	−
23	−	−	−	−	−	+	−	−
24	−	−	+	−	+	−	−	−
25	−	−	−	−	−	−	−	−
26	−	−	−	−	−	−	+	+

* Remark; No. of case in this Table I (B) corresponds to that in Table I (A).

Sato's test.

It may be worth while to mention that the cases showing fibrosis or fibrosis with changes of cytoplasm amounted to 7 in number and these were all positive to Sato's bromsulfalein test. As already reported, Sato[3] demonstrated that Sato's modification of the bromsulfalein test is sensitive enough to reveal histopathological changes in the biopsied livers in a

TABLE II (A)

Clinical Diagnosis, Total Protein and Electrophoresis of the Sera from Children with Negative Reaction to Sato's Bromsulfalein Test

No. of case	Name, sex and age (y.m.)	Dlinical diagnosis	Total protein (g. per 100cc.)	Electrophoretic components			
				Albumin (%)	Globulin (%)		
					α	β	γ
27	M.N. ♀ 2.2.	Heine-Medin's disease	6.78	50.9	15.2	15.0	18.9
28	J.K. ♂ 6.0.	Ascariasis?	7.87	57.9	11.6	14.3	16.2
29	M.M. ♀ 1.7.	Rickets	6.72	54.3	14.8	13.3	17.6
30	Y.N. ♀ 2.8.	Rickets+N.D.*	6.97	61.1	11.4	12.7	14.8
31	K.Y. ♀ 2.11.	Rickets+N.D.*	5.43	49.5	17.3	15.5	17.7
32	O.M. ♂ 4.2.	Tuberculosis of hilar lymphnodes	6.08	49.8	17.9	15.4	16.9
33	H.Y. ♂ 2.0.	Acute bronchitis	7.01	49.5	17.3	15.5	17.7
34	J.K. ♂ 4.4.	Convalescence of Izumi fever	6.06	48.2	19.5	15.8	16.5
35	A.K. ♂ 4.10.	Ascariasis	7.47	47.9	17.5	14.1	20.5

* N.D.=Nutritional dystrophy.

TABLE II (B)

Histopathological Changes in the Biopsied Livers of Children with Negative Reaction to Sato's Bromsulfalein Test

No.* of case	Infiltration of cells		Changes in cytoplasm				Fibrosis	
	Lobules	Portal tracts	Swelling	Granular appearance	Fatty granules	Fatty drops	Diffuse	Portal
27	−	−	−	−	−	−	−	−
28	−	−	+	−	−	−	−	−
29	−	−	−	−	+	−	−	−
30	−	−	−	−	−	−	−	−
31	−	−	+	−	−	−	−	−
32	−	−	−	−	−	−	−	−
33	−	−	−	−	−	−	−	−
34	−	−	−	−	−	−	−	−
35	−	+	−	−	+	−	−	−

* Remark; No. of case in Table II (B) corresponds to that in Table II (A).

degree so slight that they can not be detected by the ordinary bromsulfalein test. This opinion of Sato's is supported by the results of this investigation.

(II) Relations among electrophoretic pattern of sera, liver biopsy

TABLE III

Distribution of the Cases according to Sato's Bromsulfalein Test and Histopathological Findings of the Biopsied Liver in Sick Children

Histopathological changes in the biopsied liver	Sato's Bromsulfalein test	
	Negative	Positive
None	Case 27, " 30, " 33, " 34 } 4 cases (Group A)	Case 1, " 15, " 18, " 19, " 20, " 25 } 6 cases (Group C)
Changes of cytoplasm	Case 28, " 29, " 31, " 32, " 35 } 5 cases (Group B)	Case 2, " 3, " 4, " 6, " 7, " 8, " 13, " 16, " 17, " 21, " 22, " 23, " 24 } 13 cases (Group D)
Fibrosis	0	Case 26, " 5 } 2 cases
Changes of cytoplasm and fibrosis	0	Case 9, " 10, " 11, " 12, " 14 } 5 cases (Group E)
Total	0	26 | 35

findings and Sato's bromsulfalein test:—Total protein and electrophoretic components of sera for Groups A, B, C, D, and E are given in Table IV. The electrophoretic pattern for each Group is demonstrated diagram-

matically in Fig. 1, in which the width of column represents the intensity in histopathological changes in the biopsied livers and the length of the column represents a range from an average, (an average being indicated by a circle.) The black columns are those obtained from the cases with positive response to Sato's test and the white columns from those negative to Sato's test.

TABLE IV

Total Protein and Electrophoretic Components of Groups A, B, C, D and E and 'Normal Values' for Children

Group	Total protein g. per 100cc.	Albumin (%)	Globulin (%)		
			Alpha	Beta	Gamma
A	6.70±0.53	52.4±5.9	15.8±3.7	14.7±1.8	16.9±2.5
B	6.71±1.20	51.8±6.1	15.8±3.2	14.5±1.5	17.7±1.9
C	6.37±0.73	47.2±7.6	16.6±3.8	15.0±3.1	20.1±3.4
D	6.97±0.40	49.3±3.0	14.8±1.5	15.0±1.3	20.9±2.3
E	7.31±1.50	38.4±7.4	16.5±6.0	15.5±1.0	29.4±5.7
'Normal'	6.71±0.19	58.7±2.5	10.1±0.8	12.5±0.8	18.6±1.9

For 'normal values' of electrophoretic components for children, Yoshida,[6] one of the authors, has given the averages of 58.7±2.5%, 10.1 ±0.8%, 12.5±0.8% and 18.6±1.9% for those of albumin, α-globulin, β-globulin and γ-globulin respectively. In Fig. 1, a horizontal line which was drawn through the points at 57.8%, 10%, 12% and 18% of albumin, α-globulin, β-globulin and γ-globulin respectively, may be considered as a 'normal average-line' for electrophoretic components of Japanese children.

(a) Positive cases to Sato's bromsulfalein test (Cf. columns C, D and E in Fig. 1)

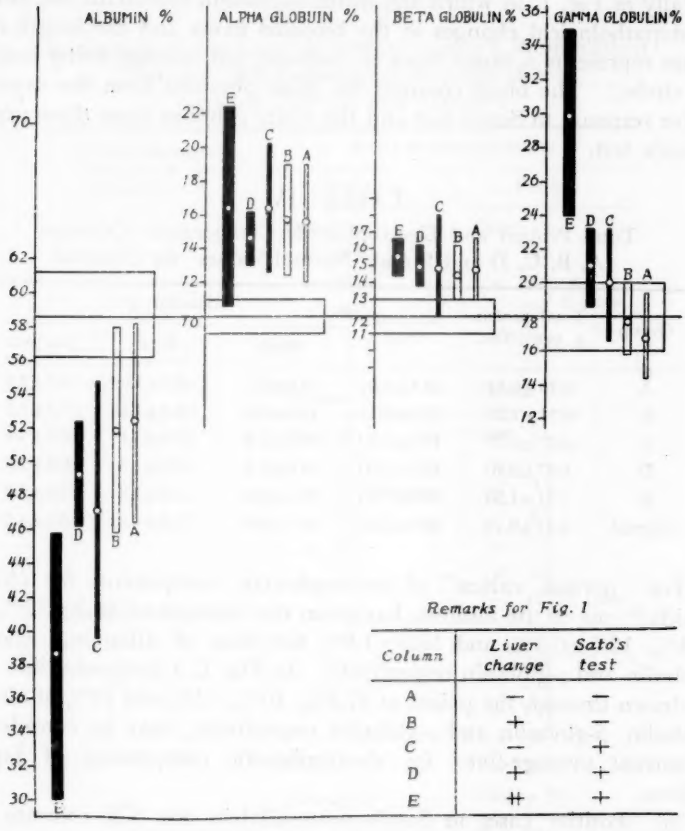
Albumin:—Averages for Groups C, D and E were found significantly below the 'normal line.' Decrease in average of albumin was demonstrated more conspicuously in Group E than in Groups C and D.

Alpha and beta globulins:—Averages for Groups C, D and E were found above the 'normal line,' but there were no significant differences among these averages compared with each other.

Gamma globulin:—The average for Group E was far from 'normal line' (with a statistical significance) and the averages for Groups Dand C were above the 'normal line' (but not signigicantly).

(b) Negative cases to Sato's bromsulfalein test (Cf. columns A and B in Fig. 1).

Albumin:—Averages for Groups A and B were below the 'normal



Remarks for Fig. 1

Column	Liver change	Sato's test
A	—	—
B	+	—
C	—	+
D	+	+
E	++	+

Fig. 1 Diagram of relations among electrophoresis of sera, liver biopsy finding and Sato's bromsulfalein test in children.

line' (but not significantly).

Alpha and beta globulins:—Averages for Groups A and B were significantly above the 'normal line.'

Gamma globulin:—Averages for both Groups A and B were within 'normal range.'

From these results it may be said that the 'abnormal' electrophoretic pattern characterized by an increase in gamma globulin accompanied by a decrease in albumin was found only for Group E. A tendency toward a decrease of albumin and an increase of gamma globulin was found to be greater in the Sato positive cases (Groups C, D, and E) than

in those with negative response to Sato's test (Groups A and B), and it was more so in the cases which were positive to Sato's test and showed histopathological alteration of the livers (Groups D and E) than in those which were positive to Sato's test but showed no pathological changes of the livers (Group C).

The finding that the averages of alpha and beta globulins were significantly increased above ' normal values ' in the majority of cases for Groups A, B, C, D, and E, may be ascribed to the fact that among the sick children subjected to the present investigation were included not a few cases with clear syndrome of nutritional dystrophy.[6]

Summary

Electrophoretic analysis of sera, liver biopsy and Sato's modified bromsulfalein test were carried out simultaneously upon the sick children, from whom were excluded those who were positive to the ordinary bromsulfalein test in which the dye was injected in an amount of 5 mg. per kg. of body weight.

According to the relations among electrophoretic pattern, liver biopsy findings and Sato's bromsulfalein test, all the cases examined were divided into 5 groups (Groups A, B, C, D, and E); Groups A and B included the negative cases to Sato's test and Groups C, D and E included the cases positive to Sato's test; histopathological changes in the livers were revealed by a needle biopsy in Groups B, D and E, and not in Groups A and C.

Histopathological changes in the biopsied livers seemed to be more frequently found in the cases positive to Sato's test (Groups C, D, and E) than in the negative cases (Groups A and B), and pathological changes of cytoplasm with fibrosis were found only in the cases with positive response to Sato's test (Group E).

Irrespective of response to Sato's bromsulfalein test and histopathological findings of the biopsied liver, a significant increase in the averages of alpha and beta globulins was found in almost all groups (Groups A, B, C, D and E) and this finding may be partly ascribed to the fact that not a few cases with clear symptoms of nutritional dystrophy were included in the patients subjected to the present investigation.

The ' abnormal ' electrophoretic pattern characterized by an increase in gamma globulin accompanied by a decrease in albumin was found in the cases which were positive to Sato's test and, in addition, showed histopathological changes of cytoplasm with fibrosis, in the biopsied livers (Group E).

A tendency toward an increase of gamma globulin as well as a decrease of albumin seemed to be very marked in the cases with histo-

pathological changes in the biopsied livers and in those with positive response to Sato's bromsulfalein test as compared with those without pathological changes in the livers or with negative response to Sato's test.

Conclusions

Among the sick children who showed negative response to the ordinary bromsulfalein test, the 'abnormal' electrophoretic pattern characterized by an increase in gamma globulin accompanied by a decrease in albumin was found in the cases which were positive to Sato's bromsulfalein test and, in addition, showed histopathological changes of cytoplasm with fibrosis in the biopsied livers.

This work was carried out by a Grant in aid for scientific developmental research of the Ministry of Education. Prof. Ts. Arakawa.

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Studies on Inhibiting Effect of Aminopterin and other Folic Acid Antagonist, and Folic Acid upon the Yoshida Sarcoma

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(Received for publication, July 26, 1952)

Since attention had been called to the significant inhibiting effect of folic acid antagonists upon malignant growth, numerous researches of their inhibiting effect were made.[1] So far some researchers have reported on this inhibiting effect of the folic acid. However, the description of morphological changes of tumor cells through this substance does not seem sufficient. In the present studies I examined chiefly cytological effect of the folic acid and folic acid antagonists upon Yoshida sarcoma cells, taking into consideration their inhibiting effect upon the tumor growth.

Substances examined are as follows:—

1) Aminopterin
2) Methopterin
3) Folic acid
4) 2,4,5,6-tetraaminopyrimidine
5) 2-amino 4-hydrooxy 6,7-dimethylpteridine
6) 2,4-diamino 6,7-dimethylpteridine
7) 2,4-diamino 7 or 6-tetraoxybutylpteridine
8) 2,6-diaminopurine
9) 2,5,6-triamino 4-oxypyrimidine
10) 2-amino 4-oxy 6 or 7-phenylpteridine
11) 2,4-diamino 6 or 7-phenylpteridine
12) 2,4-diamino 6,7 (9, 10-phenanthro) pteridine
13) 2,4-diamino 6,7-diphenylpteridine
14) 2,4-diamino 6 or 7-(*p*-acetaminophenyl) pteridine
15) 2,4-diamino 6 or 7-(*p*-aminophenyl) pteridine

The compounds were supplied by the Iatrochemical Institute Tokyo, except aminopterin and methopterin which were offered from the Lederle Laboratories through Dr. Sugiura of the Sloan-Kettering Institute, New

York City. Aminopterin supplied by the Iatrochemical Institute was also examined.

Experiments were carried out after the formula of our "Screening-test" employing Yoshida Sarcoma animals.[2)]

Results

1. Aminopterin: Table I indicates the survival days of tumor animals injected with aminopterin in different doses. Cases injected 1.0 mg., 0.5 mg., and 0.25 mg. died shortly after the injection from intoxication. In cases of 0.1 mg. injection, some died from intoxication, while others survived and died later from tumor invasion. Cases injected 0.05 mg. did not show any sign of intoxication and died from tumor invasion. Three of the four animals received a single injection of 0.05 mg. showed an evident prolongation of life (Table I).

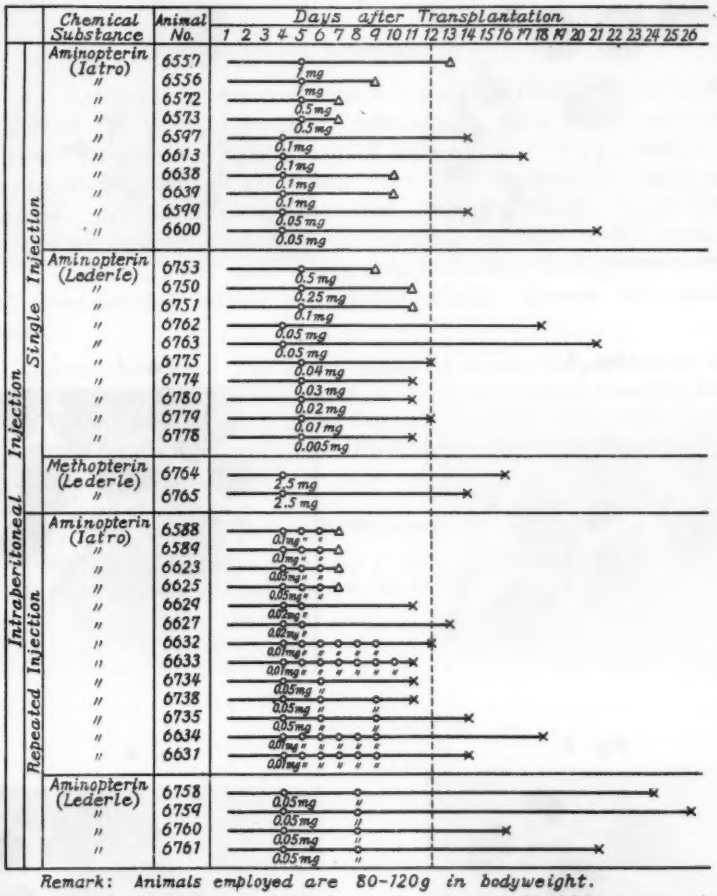
The principal changes of tumor cells are degeneration of nuclei, such as caryorrhexis, caryolysis, etc., in resting tumor cells, but changes of chromosomes in mitotic tumor cells are not evident. Though the degeneration of cytoplasma is noted in cases of 0.05 mg. injection, it is most evident in cases of 1.0 mg. injection. It begins to appear from the 12. hour after injection and comes to the extreme after the 24. hour when in the majority of tumor cells as well as in normal monocytes and eosinoophile leucocytes in the ascites nuclear destructions are noted (Fig. 1–2). The neutrophile leucocytes do not appear from immediately after injection to the end. At the 48. hour almost remaining tumor cells are remarkably increased in size (swelling) and their cytoplasma indicates vacuolar degeneration (Figs. 3–6). At the 72. hour cytoplasma of tumor cells is stained so faintly that it is very hard to distinguish them from normal monocytes. At this time epistaxis and hemorrhagic diarrhoea begin to appear and the animals die in few days after that, i.e. about on the 7th day after the injection.

In case of 0.05 mg. injection, the degeneration of tumor cells appears slowly from the 24. hour. At the 72. hour, however, the peritoneal cavity has regained the pure culture state of actively proliferating tumor cells.

In cases of 0.03 mg., 0.02 mg., 0.01 mg., and 0.005 mg. injections the changes in tumor cells did not occur.

Since aminopterin is fairly toxic, it seems to expect any prolongation of life of tumor animals by the injection of this substance. However, in three of the four cases which received the twice repeated injection of 0.05 mg. aminopterin (Lederle) remarkable prolongation of life (Table I) was obtained. In many cases which received doses 0.1 mg. anemia, epistaxis, and hemorrhagic diarrhoea appeared about 4 days after injection.

TABLE I



Remark: Animals employed are 80-120g in bodyweight.

------- Average survival days of untreated Yoshida sarcoma animals

× Death from tumor invasion △ Death from intoxication

Subcutaneous injection of the substance presented the almost same cytological effect as intraperitoneal injection upon the tumor cells in the ascites.

2. Methopterin: Injection of the substance 2.5 mg. (Lethal dose is 5 mg. per 100 g. body weight) produced the almost same cellular changes as above described.

3. Twelve other folic acid antagonists and folic acid demonstrated

Fig. 1

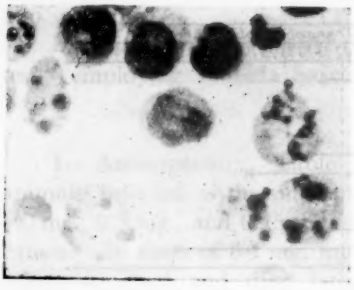


Fig. 2

Fig. 3

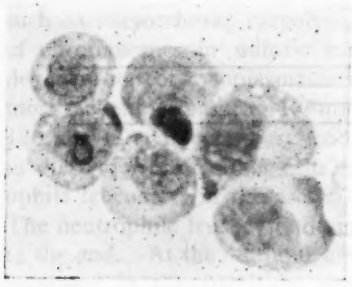


Fig. 4

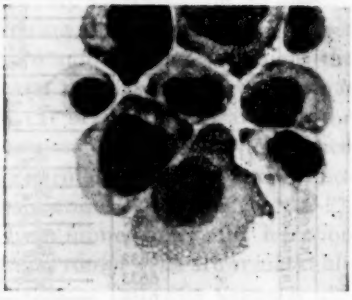
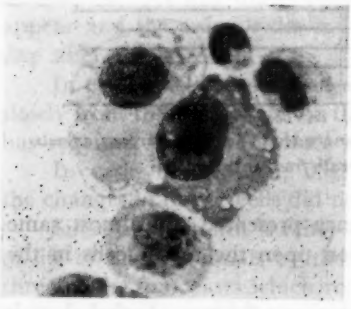
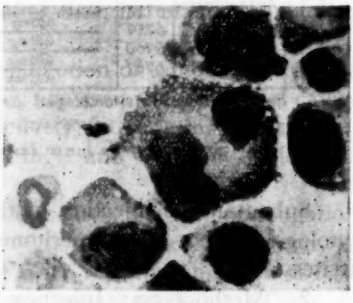


Fig. 5



Gig. 6



Figs. 1–2. 2 days after injection of 1.0 mg. aminopterin: Caryorrhexis of tumor cells.

Figs. 3–6. 2 days after injection of 1.0 mg. aminopterin: Swelling and vacuolar degeneration of cytoplasma, and faintly stained nuclei.

neither notable cellular changes nor inhibiting effect even in cases given the half lethal dose or the lethal dose.

Summary

1. Aminopterin demonstrated a notable inhibiting effect upon the growth of the Yoshida sarcoma, especially in cases which received the repeated intraperitoneal injection of 0.05 mg. per 100 g. body weight. Non-specific changes in the tumor cells after the injection, such as caryorrhexis and vacuolar degeneration were described. These cellular changes were obtained also by subcutaneous injection of the compound.

2. Other 13 folic acid antagonists including Methopterin and folic acid demonstrated neither remarkable cellular changes nor inhibiting effect.

Acknowledgements. The author wishes to express his gratefulness to the Lederle Laboratories, Dr. Sugiura, and the Iatrochemical Institute for supplying the compounds which made this study possible. This study was supported by the "Grant in Aid for Fundamental Scientific Research" of the Ministry of Education.

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Effect of Cacodylic Acid upon the Yoshida Sarcoma

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(Received for publication, July 26, 1952)

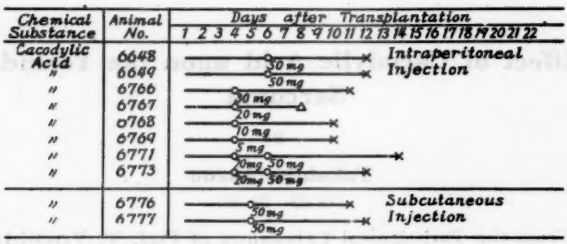
Since 1937 cacodylate has been well known as having a colchicine effect by the study of Dustin and his collaborators,[1] so that I examined the effect of cacodylic acid upon the Yoshida sarcoma to ascertain whether it has a colchicine effect or not.

Experiment was carried out after the formula of our " Screening-test " employing Yoshida sarcoma animals.[2]

Table I indicates the survival days of tumor animals injected with cacodylic acid solution in different doses. The lethal dose of cacodylic acid is 100 mg. per 100 g. body weight. Cases injected intraperitoneally 50 mg. and 30 mg. indicate the same cellular changes upon tumor cells. The principal changes of tumor cells caused after injection of cacodylic acid are the scattering of lacerated chromosomes and the arrest of mitosis in the metaphase. These changes begin to come out shortly after injection and come to the extreme after 12. hour. At 1. hour the remarkable scattering of lacerated chromosomes and the increase of mitosis in number are found in tumor ascites and at 3. hour come to high degree (Figs. 1–2). At 6. and 9. hour the scattering of lacerated chromosomes and the increase of mitosis in number come increasingly to high degree and at the same time cytolysis and vacuolar degeneration of nuclei are found in resting tumor cells (Figs. 3–4). At 12. hour the increase of mitosis in number comes to the highest degree, the majority of mitotic tumor cells is in the metaphase, and the anaphase and the telophase are not found (Fig. 5). Therefore it may safely be said that mitosis is arrested in the metaphase. At 24. hour there are not the changes in mitotic tumor cells, but there are many polynucleate tumor cells in the tumor ascites (Fig. 6). Polynucleate tumor cells are found also at 72. hour after injection, but after all at this time the abdominal cavity has regained the pure culture state

* This research owes to the " Grant in Aid for Fundamental Scientific Research " of Ministry of Education.

TABLE I



Note: × Death from tumor invasion

△ Died of pneumonia

TABLE II

The Number of Mitosis in each Phase after Injection of Cacodylic Acid 50 mg. (No. 6648)

	Before inj.	After injection				
		1 h.	3 hs.	6 hs.	9 hs.	12 hs.
Prophase	20	17	5	17	16	17
Metaphase	21	221	228	351	523	596
Anaphase	7	5	2	0	0	0
Telophase	21	0	0	0	0	0
Total number of mitosis	69	243	235	368	539	613

TABLE III

The Percentage of Mitosis in each Phase (No. 6648)

Figures express Percentages

	Before inj.	After injection				
		1 h.	3 hs.	6 hs.	9 hs.	12 hs..
Prophase	29.0	6.9	2.1	4.6	2.9	2.7
Metaphase	30.4	91.1	97.2	95.4	97.1	97.3
Anaphase	10.2	2.0	0.7	0	0	0
Telophase	30.4	0	0	0	0	0

of tumor cells.

Cacodylate is well known as having a colchicine effect. It becomes clear if above-mentioned is explained mathematically, so I counted the number of mitosis among 5,000 tumor cells after intraperitoneal injection of cacodylic acid 50 mg. and classified them into each phases (Tables II and III). The number of mitosis in tumor ascites before injection

Fig. 1

Fig. 2

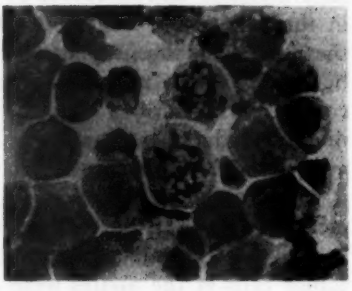


Fig. 3

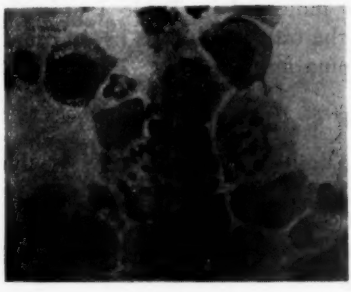


Fig. 4

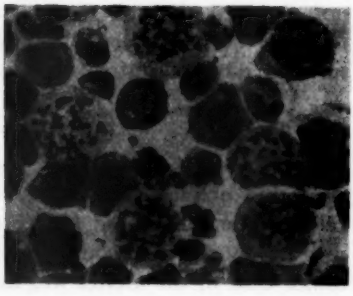


Fig. 5

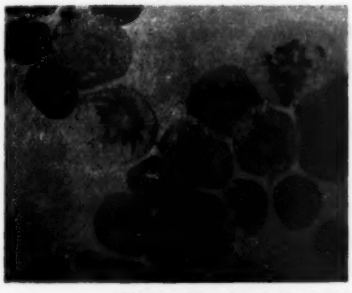
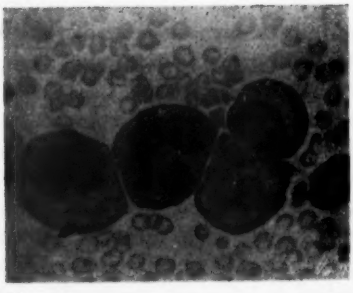


Fig. 6



Figs. 1–2. After three hours from injection.

Fig. 3. After six hours from injection: Scattering of lacerated chromosomes.

Fig. 4. After nine hours from injection.

Fig. 5. After twelve hours from injection: The majority of mitotic tumor cells are in the metaphase and caryolysis in resting tumor cells.

Fig. 6. Twenty four hours after injection: There are many polynucleate tumor cells.

was 69, but it increased 221 at 1. hour, 228 at 3. hour, and 596 at 12. hour after injection and moreover at this time more than 90% of mitosis were in the metaphase. In Tables II and III there was not a difference in the number of the prophase between before injection and after injection, but there was a remarkable difference in percentage. Dustin and his colaborators described that cacodylate promotes at the first stage a cell division and the increase of mitosis in number is based upon it. However, I could not find from Tables II and III that cacodylic acid promotes a cell division at the first stage. From above-mentioned, it becomes clear that mitosis is arrested in the metaphase and does not advance forward.

In case of subcutaneous injection the above-described changes of tumor cells in the ascites were not evident.

Though it was confirmed that cacodylic acid has a typical colchicine effect upon Yoshida sarcoma cells, the inhibition of tumor growth was not obtained through this substance. As indicated in Table I animals received the substance in 10–14 days after the transplantation, i.e. in usual survival days of untreated Yoshida sarcoma animals.

Conclusion

1. Action of cacodylic acid upon Yoshida sarcoma cells is the scattering of lacerated chromosomes and the arrest of mitosis of tumor cells in the metaphase (Colchicine effect).
2. By subcutaneous injection of the substance the effect upon the tumor cells in the ascites was not evident.
3. Inhibiting effect of cacodylic acid upon the Yoshida sarcoma was not presented.

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Studies on Blood and Plasma Catalase

The 4th Report

The Blood and Plasma Catalase Content in Various Experimental Anemias

By

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(Received for publication, July 30, 1952)

From our former report[1], it is presumed that the blood catalase content changes in the close connection with erythrocytes and hemoglobin, but the plasma catalase content is influenced not only by the hemopoietic function, but also by the hemolysis in a living body and the hemoclastic function of the spleen.

Therefore, we made an attempt to measure the blood and plasma catalase content in various experimental anemias to make clear its significance.

Experimental

Results

1. Anemia caused by artificial bleeding

In our former report[2] we confirmed that there is no evident diurnal variation of the blood and plasma catalase content as the control test of normal rabbits.

In order to make an artificial anemia, 10 cc. of the blood was taken out from rabbits for 7 days by heart puncture. Data presented in Table I show that the blood catalase content decreases in company with erythrocytes and hemoglobin in anemia caused by artificial bleeding, but the recovery of the blood catalase content after stopping the bleeding precedes that of erythrocytes and hemoglobin, while the variation of the plasma catalase content is not remarkable. Moreover, the catalase index, hemoglobin-catalase index and plasma-blood catalase ratio tend to increase temporarily.

2. Anemia caused by phenylhydrazine

After subcutaneous injection of phenylhydrazine hydrochloride in doses of 2 cc. per kg. body weight of 1% solution for 3 days, the blood

TABLE I

Blood and Plasma Catalase Content by Artificial Bleeding

	No.	Before bleeding	1st day after bleeding	2nd " "	3rd " "	4th " "	5th " "	6th " "	7th " "	8th " "	9th " "	11th " "	13th " "	15th " "
Erythrocytes	1	614	592	407	336	348	471	445	443	420	420	481	534	608
	2	518	474	411	330	342	312	242	287	303	336	346	486	580
Hb.	1	86	70	55	58	51	50	50	45	44	59	65	72	81
	2	88	82	75	55	58	55	53	53	55	52	57	68	77
Hb-index	1	0.70	0.59	0.68	0.86	0.73	0.53	0.56	0.51	0.52	0.71	0.68	0.68	0.67
	2	0.85	0.86	0.92	0.83	0.85	0.88	1.09	0.92	0.91	0.77	0.82	0.70	0.66
Blood catalase	1	5.64	5.10	4.86	3.91	3.91	4.93	5.81	4.25	4.01	4.79	4.79	7.14	6.09
	2	7.21	4.69	3.74	4.35	4.01	3.20	3.84	3.60	4.42	3.81	4.96	5.10	5.27
Plasma catalase	1	25.16	13.60	17.00	29.24	13.60	5.44	23.46	25.16	11.56	10.40	4.76	30.60	34.68
	2	26.52	10.20	12.24	8.84	23.80	12.24	19.04	29.92	16.64	17.68	27.20	8.16	25.84
Catalase index	1	0.92	0.86	1.19	1.16	1.12	1.05	1.31	0.96	0.96	1.14	1.00	1.33	1.00
	2	1.39	0.99	0.91	1.32	1.17	1.02	1.58	1.26	1.46	1.13	1.43	1.05	0.91
Hb-catalase index	1	0.38	0.43	0.51	0.39	0.44	0.59	0.67	0.55	0.53	0.47	0.43	0.57	0.44
	2	0.48	0.33	0.29	0.46	0.40	0.34	0.42	0.40	0.46	0.43	0.51	0.44	0.40
Plasma-blood catalase ratio	1	4.46	2.67	3.49	7.47	3.47	1.11	4.03	5.91	2.88	2.12	0.99	4.29	5.71
	2	3.68	2.18	3.27	2.03	5.94	3.81	4.96	8.27	3.76	4.63	5.52	1.60	4.90

catalase content decreased rapidly together with erythrocytes and hemoglobin, but their recovery differed as presented in Table II. On the contrary, the plasma catalase content increased immediately after phenylhydrazine as well as reflected image in the mirror, and returned to a normal extent very slowly after stopping the injection.

3. Anemia caused by saponin

When 1% saponin was intravenously injected in doses of 1 cc. per kg. on the first day, 1.5 cc. on the second day and 2 cc. on the third day, the blood catalase content decreased in a slight degree in company with erythrocytes and hemoglobin, contrary to the markedly increased content of plasma catalase, and then the recovery of the blood catalase content and hemoglobin preceded that of erythrocytes (Table III).

4. Anemia caused by colloidal silver

The results presented in Table IV show that 1% colloidal silver used intravenously in doses of 2 cc. per kg. for 20 days lowers little by little the blood catalase content, erythrocytes and hemoglobin, but the decrease of the blood catalase content takes place newly later after stopping the injection. In particular, it is notable that the plasma catalase content increases in the first stage, but decreases afterwards preceding that of the blood catalase.

5. Intoxication by sodium nitrite

When 0.5 cc. per kg. of 10% sodium nitrite which produces methemo-

TABLE II

Blood and Plasma Catalase Content by Phenylhydrazine Injection

	No.	Before injection	1st day		2nd day		3rd day		4th day after injection	5th "	7th "	9th "	13th "	17th "	21th "	28th "	35th "
			Before injection	3h after injection	Before injection	3h after injection	Before injection	3h after injection									
Erythrocytes	1	609	735	597	552	514	394	350	272	179	308	393	536	537	635	554	670
	2	706	671	631	634	579	425	397	232	149	139	240	467	560	553	659	604
Hb.	1	86	85	83	80	70	48	40	32	44	60	76	88	95	96	87	96
	2	100	100	93	75	68	55	45	23	23	30	51	80	85	112	105	107
Hb-index	1	0.71	0.58	0.69	0.73	0.68	0.62	0.57	0.61	1.23	0.97	0.97	0.82	0.88	0.76	0.78	0.72
	2	0.71	0.75	0.74	0.59	0.59	0.65	0.56	0.50	0.77	1.11	1.06	0.86	0.71	1.01	0.80	0.88
Blood catalase	1	4.91	4.25	4.52	4.42	4.28	3.57	3.50	2.72	3.88	6.05	7.75	7.58	7.69	7.17	6.94	7.04
	2	4.91	4.62	4.86	5.13	4.08	3.13	2.92	2.18	1.94	2.79	5.30	7.14	6.66	7.24	6.80	6.73
Plasma catalase	1	12.35	8.16	10.20	68.68	95.88	74.46	102.68	127.84	58.48	36.72	16.32	17.00	11.56	4.76	10.88	17.68
	2	21.86	13.94	27.52	40.80	40.80	44.88	73.44	74.80	69.02	68.68	40.80	21.76	10.20	17.68	4.08	12.92
Catalase index	1	0.80	0.58	0.71	0.80	0.83	0.90	1.00	1.00	2.16	1.97	1.97	1.41	1.43	1.13	1.25	1.05
	2	0.69	0.67	0.77	0.81	0.70	0.74	0.74	0.94	1.31	2.01	2.21	1.53	1.19	1.31	1.03	1.11
Hb-catalase index	1	0.33	0.29	0.32	0.32	0.36	0.43	0.51	0.49	0.51	0.59	0.59	0.50	0.47	0.43	0.46	0.43
	2	0.28	0.27	0.31	0.40	0.34	0.33	0.38	0.54	0.49	0.54	0.63	0.52	0.46	0.37	0.37	0.37
Plasma-blood catalase ratio	1	2.52	1.92	2.26	15.51	22.40	20.82	29.30	46.80	15.08	6.07	2.11	2.24	1.50	0.66	1.57	2.51
	2	4.46	3.02	5.65	7.94	10.00	14.34	25.10	34.30	35.50	24.60	7.71	3.05	1.53	2.44	0.60	1.92

TABLE III

Blood and Plasma Catalase Content by Saponin Injection

	No.	Before injection	1st day		2nd day		3rd day		4th day after injection	5th " "	7th " "	10th " "	13th " "	16th " "
			Before injection	3h after injection	Before injection	3h after injection	Before injection	3h after injection						
Erythrocytes	1	597	593	617	452	424	494	461	392	407	413	463	424	479
	2	695	697	676	594	659	686	620	574	587	587	590	544	596
Hb.	1	79	83	94	85	78	64	52	58	55	58	75	72	65
	2	98	99	107	110	110	94	90	94	86	92	92	83	90
Hb-index	1	0.66	0.70	0.76	0.94	0.92	0.65	0.57	0.74	0.68	0.70	0.81	0.86	0.68
	2	0.70	0.72	0.79	0.93	0.84	0.64	0.73	0.82	0.73	0.79	0.78	0.76	0.76
Blood catalase	1	7.41	7.75	6.83	7.45	6.77	8.02	5.41	6.56	6.09	6.39	8.30	9.25	8.02
	2	7.41	7.41	6.49	7.92	6.97	8.19	7.58	7.31	6.66	6.80	7.58	9.09	8.19
Plasma catalase	1	17.68	8.16	12.24	10.20	47.60	61.24	53.04	40.12	36.04	21.08	12.92	21.76	13.60
	2	11.56	8.16	29.24	9.52	40.12	30.60	66.64	54.40	50.32	36.72	19.72	19.72	12.24
Catalase index	1	1.24	1.31	1.11	1.65	1.59	1.62	1.17	1.69	1.49	1.55	1.79	2.17	1.67
	2	1.06	1.06	0.96	1.33	1.06	1.19	1.22	1.27	1.13	1.16	1.28	1.67	1.38
Hb-catalase index	1	0.55	0.54	0.42	0.51	0.50	0.73	0.60	0.66	0.64	0.64	0.64	0.74	0.72
	2	0.44	0.44	0.35	0.42	0.37	0.51	0.49	0.45	0.45	0.43	0.48	0.64	0.53
Plasma-blood catalase ratio	1	2.39	1.05	1.79	1.39	7.03	7.63	9.81	6.61	5.92	3.30	1.56	2.36	1.70
	2	1.56	1.10	4.51	1.20	5.77	4.40	8.79	7.44	7.54	5.40	2.60	2.17	1.49

TABLE IV

Blood and Plasma Catalase Content by Colloidal Silver Injection

	No.	Before injection	1st day after injection	3rd " "	5th " "	7th " "	9th " "	11th " "	13th " "	17th " "	21th " "	25th " "	29th " "	35th " "	40th " "	50th " "	60th " "	70th " "
Erythrocytes	1	616	729	468	530	522	516	532	486	507	446	430	458	518	570	540	556	560
	2	618	575	644	682	544	565	540	562	424	520	311	529	527	600	630	650	649
Hb.	1	80	98	63	68	75	65	66	64	55	63	57	63	74	80	80	82	84
	2	85	82	87	80	77	75	68	70	62	58	56	62	71	86	88	98	100
Hb-index	1	0.65	0.67	0.67	0.64	0.68	0.63	0.62	0.66	0.55	0.71	0.66	0.70	0.71	0.74	0.74	0.74	0.75
	2	0.69	0.71	0.68	0.59	0.71	0.65	0.63	0.62	0.73	0.56	0.90	0.59	0.62	0.72	0.70	0.75	0.77
Blood catalase	1	5.68	5.13	3.77	3.98	4.93	4.96	4.83	3.54	5.24	4.96	5.71	5.34	4.79	5.30	4.42	3.33	3.13
	2	5.61	5.51	4.59	5.75	5.51	5.00	4.90	4.69	4.86	5.20	5.30	4.83	5.07	5.61	5.10	3.88	3.94
Plasma catalase	1	47.94	35.36	23.12	31.28	43.52	45.56	58.48	30.60	26.52	53.04	34.00	38.76	18.36	23.12	10.54	6.80	7.48
	2	34.00	23.80	12.24	47.60	51.68	44.88	49.64	39.44	42.84	55.08	34.68	22.44	53.72	23.80	13.60	7.48	10.20
Catalase index	1	0.92	0.70	0.81	0.75	0.89	0.96	0.91	0.74	1.03	1.11	1.33	1.16	0.92	0.93	0.82	0.60	0.56
	2	0.99	0.89	0.71	0.84	1.01	0.89	0.91	0.83	1.14	1.00	1.70	0.91	0.96	0.93	0.81	0.60	0.61
Hb-catalase index	1	0.41	0.30	0.35	0.34	0.38	0.44	0.43	0.32	0.53	0.46	0.58	0.49	0.38	0.38	0.32	0.24	0.22
	2	0.38	0.39	0.31	0.42	0.41	0.39	0.42	0.39	0.45	0.52	0.55	0.45	0.41	0.38	0.34	0.23	0.23
Plasma-blood catalase ratio	1	8.29	6.88	8.64	7.85	8.82	9.17	12.08	8.67	5.07	10.70	5.96	7.24	3.82	4.34	2.38	2.04	2.39
	2	6.06	4.32	2.67	8.28	9.39	8.98	10.11	8.40	8.81	10.57	6.55	4.64	10.71	4.24	2.67	1.93	2.59

TABLE V

Blood and Plasma Catalase Content by Sodium Nitrite Injection

	No.	Before injection	After injection (time in hours)						
			0.25	0.5	1	2	3	5	7
Erythrocytes	1	617	575	614	599	617	577	629	615
	2	621	618	623	598	624	608	628	619
Hb.	1	97	92	101	104	102	95	102	94
	2	90	90	91	95	98	92	101	91
Hb-index	1	0.78	0.80	0.82	0.87	0.83	0.82	0.81	0.76
	2	0.73	0.73	0.73	0.79	0.79	0.75	0.80	0.74
Blood catalase	1	8.87	8.84	8.53	7.75	7.99	8.60	8.06	7.99
	2	8.02	8.19	7.75	7.58	8.30	7.96	7.75	7.99
Plasma catalase	1	27.88	19.04	19.38	23.80	18.36	23.12	20.74	18.02
	2	13.60	12.24	12.58	17.68	11.56	14.62	12.92	11.22
Catalase index	1	1.44	1.54	1.38	1.29	1.29	1.47	1.28	1.30
	2	1.29	1.32	1.24	1.27	1.32	1.30	1.23	1.29
Hb-catalase index	1	0.53	0.56	0.49	0.43	0.46	0.52	0.46	0.49
	2	0.52	0.53	0.49	0.47	0.49	0.50	0.45	0.51
Plasma-blood catalase ratio	1	3.14	2.16	2.27	3.07	2.30	2.69	2.57	2.25
	2	1.70	1.40	1.62	2.33	1.39	1.84	1.67	1.40

globin in the blood, was intravenously injected, the change of the blood and plasma catalase content, erythrocytes and hemoglobin remained almost within a physiological variation (Table V).

Discussion

It is generally accepted that the blood catalase content decreases in parallel with erythrocytes and hemoglobin in anemia caused by artificial bleeding (Bernstein[3], Ochi[4], Nozaki[5], Takimoto and Kubo[6], Bode[7], v. Krüger[8], Fujikawa[9]), but Kurokawa[10] and Takisawa[11] recognize the plasma catalase content to be unchangeable. As for the catalase index of the above-stated anemia, Nozaki[5], Takimoto[6] and Fujikawa[9] recognize the temporary increase, but Bode[7], Nissen[12], Bernstein[3], Ochi[4] and v. Krüger[8] report it to be constant. The results obtained from our experiments show that the blood catalase content decreases in company with erythrocytes and hemoglobin, but the recovery of the former precedes that of erythrocytes and hemoglobin. Therefore, the catalase index, hemoglobin-catalase index and plasma-blood catalase ratio tend to increase temporarily, while the plasma catalase content is not so variable.

In anemia cuased by phenylhydrazine which is compared to pernicious anemia, many workers, such as Nissen[12], Bernstein[3], Ochi[4], Nozaki[5], Takimoto and Kubo[6], v. Krüger[8] and Fujikawa[9], recognize that the catalase index increases remarkably. Data presented in our experiments

show that the decrease of the blood catalase content precedes that of erythrocytes and hemoglobin and the increase of the plasma catalase content evidently continues for more than one week after the last injection. Therefore, the catalase index, hemoglobin-catalase index and plasma-blood catalase ratio increase remarkably, but the latter subsequently decreases to the subnormal degree.

Gaisböck and Bayer[13] as well as Kano[14] report that saponin, the hemolytic toxin, is accompanied by the disturbed function of the bone marrow. According to our results, it is presumed that the plasma catalase is produced by hemolysis in a living body, because it changes as reflected image in the mirror, compared with the blood catalase. Moreover, in the above-stated anemia as well as anemia caused by phenylhydrazine, the recovery of the blood catalase and hemoglobin is more marked than that of erythrocytes, therefore, it is accompanied by the excitement of the hemopoietic function.

As to the blood and plasma catalase of anemia caused by colloidal silver which is compared to aplastic anemia by Komiya[15], there is no report. Our results show that the decrease of the blood and plasma catalase content is still remarkable after the stopping of the injection, especially, the latter is more marked than the former.

Furthermore, we attempted to estimate the influence of the intoxication by sodium nitrite, which is recognized as a toxin to cause methemoglobin by Loewy[16]. Our results show that the variation of the blood and plasma catalase content remains almost within a physiological change, and then the catalytic power of the blood and plasma can not be identified with hemoglobin.

Therefore, it is presumed that the variation of the blood and plasma catalase content in various experimental anemias is produced by functional or anatomical disorders of the hemopoietic organs.

Summary

From these experiments with various experimental anemias caused by artificial bleeding, phenylhydrazine, saponin, colloidal silver and sodium nitrite, it can be concluded that the blood and plasma catalase is produced in the hemopoietic organs by different process from that of erythrocytes and hemoglobin, while a larger portion of the plasma catalase is produced by the hemolysis in a living body.

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Studies on Blood and Plasma Catalase

The 5th Report

Influence of the Spleen and Reticulo-Endothelial System upon the Blood and Plasma Catalase Content

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At present it is generally recognized that the spleen is an important organ not only among the hemopoietic system, but also in the reticulo-endothelial system (RES). But there is no concordant opinion as to the relation between the function of the spleen and the blood catalase. Furthermore, there has been no attempt made concerning the influence of the function of the RES upon the catalase in the blood and plasma.

According to our former report[1], it is concluded that the spleen and other hemopoietic organs have a close connection with the creation of the catalase in the blood and plasma.

Therefore, the following experiments were performed in order to clarify the relation of the spleen and RES with the blood and plasma catalase.

Experimental

Results

A. Influence of the spleen upon the blood and plasma catalase content

1. Influence of the splenectomy

In the control test of normal rabbits we confirmed that there is not only evident diurnal variation of the blood and plasma catalase content as in the former report[2], but also it is not influenced by repeated test in short intervals as presented in Table I. Data presented in Table II show that the plasma catalase content decreases up to one sixth, while erythrocytes, hemoglobin and the blood catalase content change almost within a physiological variation, when the spleen is removed from a normal rabbit.

TABLE I
Control Test

	No.	Before injection	After injection (time in hours)						
			0.25	0.5	1	2	3	5	8
Erythrocytes	1	680	685	673	677	666	681	683	669
	2	644	649	660	664	649	652	656	646
Hb.	1	93	93	90	91	91	92	93	91
	2	80	81	81	84	82	80	83	81
Hb-index	1	0.71	0.71	0.67	0.67	0.68	0.68	0.71	0.68
	2	0.62	0.62	0.61	0.63	0.63	0.62	0.63	0.63
Blood catalase	1	4.83	4.76	4.76	4.86	4.79	4.73	4.83	4.79
	2	4.59	4.56	4.59	4.66	4.62	4.56	4.66	4.56
Plasma catalase	1	29.24	29.24	26.52	28.90	26.18	28.56	29.24	29.58
	2	24.14	23.80	24.48	23.12	24.14	23.80	23.46	23.80
Catalase index	1	0.71	0.69	0.71	0.72	0.72	0.69	0.71	0.71
	2	0.71	0.70	0.70	0.69	0.71	0.70	0.70	0.71
Hb-catalase index	1	0.30	0.30	0.31	0.31	0.31	0.30	0.30	0.31
	2	0.33	0.33	0.33	0.32	0.33	0.33	0.32	0.33
Plasma-bood catalase ratio	1	6.07	6.16	5.57	5.96	5.47	6.03	6.08	6.17
	2	5.25	5.23	5.33	4.97	5.21	5.23	5.06	5.23

2. Influence of the spleen extract

When 10 cc. of the spleen extract, inactivated by heating at 60°C for 30 minutes, is subcutaneously injected in normal rabbits, erythrocytes, hemoglobin and the blood catalase decrease remarkably, contrary to the increase of the plasma catalase content (Table III). But the results from our experiments show that the above-mentioned efficiency of the spleen extract is not revealed by splenectomized rabbits (Table IV).

B. Influence of the RES upon the blood and plasma catalase content

1. Influence of the stimulation of the RES

In order to stimulate the RES, 0.3 cc. per kg. of 1% congo-red solution was intravenously injected. Data presented in Table V show that the plasma catalase content evidently increased after 3 hours, while red cells, hemoglobin and the blood catalase content decreased slightly. In the similar way, the intravenous injection of 10 cc. of 2% Japanese ink caused the increase of the plasma catalase content, as shown in Table VI.

2. Influence of the injury of the RES

When the RES was blockaded by means of the intravenous injection of 5 cc. per kg. of 3% Japanese ink, the plasma catalase content decreased to a certain extent, while erythrocytes, hemoglobin and the blood catalase content remained within a normal value (Table VII).

As already reported, the intravenous injection of 2 cc. per kg. of

TABLE II

Blood and Plasma Catalase Content by Splenectomy

	No.	Before splenectomy	1st day after splenectomy	2nd " "	3rd " "	4th " "	5th " "	6th " "	7th " "	10th " "	14th " "	18th " "	21th " "	29th " "	36th " "	43th " "	50th " "
Erythrocytes	1	669	755	670	701	692	637	636	611	749	737	668	704	739	743	703	621
	2	649	594	587	603	587	536	565	571	583	649	615	686	718	747	631	586
Hb.	1	91	93	90	85	96	90	88	91	96	86	85	90	105	92	101	89
	2	81	75	77	68	78	76	75	84	82	81	80	81	92	83	82	85
Hb-index	1	0.68	0.62	0.67	0.61	0.69	0.71	0.69	0.74	0.64	0.58	0.64	0.64	0.71	0.62	0.72	0.72
	2	0.62	0.63	0.66	0.57	0.66	0.71	0.66	0.73	0.71	0.62	0.65	0.59	0.64	0.56	0.65	0.72
Blood catalase	1	4.77	4.22	4.59	4.69	4.76	4.56	4.86	5.47	4.59	4.45	4.25	4.11	4.59	4.86	3.88	3.81
	2	4.59	3.64	3.70	3.98	4.28	3.94	4.52	5.47	4.69	4.39	3.57	4.15	4.56	5.20	4.01	3.79
Plasma catalase	1	29.54	17.00	8.16	8.16	6.12	7.48	4.76	11.56	5.44	9.52	5.44	4.08	10.26	10.20	11.56	6.80
	2	24.14	16.32	12.92	12.92	19.72	10.20	5.44	9.04	8.84	8.16	6.46	9.52	14.96	10.20	11.56	15.64
Catalase index index	1	0.71	0.56	0.69	0.67	0.69	0.72	0.76	0.89	0.61	0.60	0.63	0.58	0.62	0.65	0.55	0.61
	2	0.71	0.61	0.63	0.66	0.73	0.73	0.80	0.96	0.80	0.68	0.58	0.60	0.63	0.69	0.64	0.65
Hb-catalase	1	0.30	0.26	0.30	0.32	0.29	0.29	0.32	0.35	0.28	0.30	0.29	0.26	0.25	0.31	0.22	0.25
	2	0.33	0.28	0.28	0.34	0.32	0.30	0.35	0.38	0.33	0.32	0.26	0.30	0.29	0.36	0.28	0.26
Plasma-blood catalase ratio	1	6.20	4.03	1.78	1.74	1.29	1.64	0.98	2.11	1.19	2.14	1.28	0.99	2.24	2.10	2.99	1.79
	2	5.27	4.50	3.50	3.25	4.61	2.58	1.21	1.65	1.89	1.86	1.81	2.30	3.28	1.96	2.88	4.13

TABLE III

Influence of the Spleen Extract upon Blood and Plasma Catalase Content in Normal Rabbits

	No.	Before injection	After injection (time in hours)									
			0.5	1	2	3	4	6	12	24	48	72
Erythrocytes	1	622	610	620	615	583	585	588	521	587	591	546
	2	577	580	530	550	478	491	460	445	542	503	488
Hb.	1	87	88	90	91	91	86	78	79	84	90	83
	2	85	84	73	75	68	66	64	65	76	70	67
Hb-index	1	0.70	0.72	0.73	0.74	0.78	0.73	0.66	0.76	0.71	0.76	0.76
	2	0.74	0.72	0.69	0.68	0.71	0.67	0.70	0.73	0.70	0.70	0.69
Blood catalase	1	7.72	7.21	7.24	7.58	7.21	6.90	5.58	6.02	6.70	6.26	6.02
	2	6.70	6.66	5.68	5.54	5.41	5.34	5.13	5.10	4.49	4.49	4.66
Plasma catalase	1	8.84	11.56	10.88	6.80	36.04	22.44	13.60	5.44	6.12	5.44	10.88
	2	9.52	8.84	4.76	25.84	16.32	19.02	8.16	5.24	4.76	8.84	10.20
Catalase index	1	1.24	1.18	1.17	1.23	1.23	1.18	0.95	1.16	1.14	1.06	1.10
	2	1.16	1.15	1.07	1.00	1.13	1.09	1.11	1.14	0.83	0.91	0.95
Hb-catalase index	1	0.51	0.47	0.47	0.48	0.46	0.47	0.42	0.44	0.46	0.40	0.42
	2	0.46	0.46	0.45	0.43	0.46	0.51	0.47	0.46	0.34	0.38	0.40
Plasma-blood catalase ratio	1	1.15	1.60	1.50	0.90	5.00	3.25	2.44	0.92	0.92	0.88	1.81
	2	1.42	1.33	0.84	4.66	3.02	3.56	1.59	1.03	1.06	1.97	2.20

TABLE IV

Influence of the Spleen Extract upon Blood and Plasma Catalase Content in Splenectomized Rabbits

	No.	Before splenectomy	3rd day after splenectomy	After injection (time in hours)						
				0.5	1	2	3	4	6	12
Erythrocytes	1	618	620	616	619	598	588	569	585	566
	2	451	495	504	450	476	502	500	523	511
Hb.	1	89	88	90	92	89	86	83	86	87
	2	60	62	60	58	68	66	65	67	64
Hb-index	1	0.72	0.71	0.73	0.74	0.74	0.73	0.73	0.74	0.74
	2	0.66	0.68	0.60	0.64	0.71	0.66	0.65	0.64	0.64
Blood catalase	1	7.21	7.58	7.24	6.90	7.21	7.00	6.70	6.80	6.66
	2	6.24	5.37	5.13	5.13	5.44	5.71	6.02	5.81	5.78
Plasma catalase	1	32.64	12.58	12.92	11.90	11.90	15.30	13.60	10.20	11.22
	2	23.80	9.52	9.86	9.18	9.18	13.26	10.88	8.84	9.52
Catalase index	1	1.16	1.22	1.17	1.11	1.20	1.19	1.17	1.16	1.18
	2	1.38	1.08	1.02	1.14	1.14	1.14	1.20	1.11	1.13
Hb-catalase index	1	0.47	0.50	0.47	0.44	0.47	0.47	0.47	0.46	0.44
	2	0.61	0.50	0.50	0.51	0.47	0.51	0.54	0.51	0.52
Plasma-blood catalase ratio	1	4.53	1.65	1.78	1.73	1.65	2.18	2.03	1.50	1.68
	2	3.82	1.77	1.92	1.78	1.68	2.32	1.81	1.52	1.64

TABLE V

Blood and Plasma Catalase Content by 1% Congo-red Injection

	No.	Before injection	After injection (time in hours)							
			0.5	1	2	3	4	5	6	8
Erythrocytes	1	650	580	576	503	552	557	565	586	601
	2	579	513	512	438	466	481	477	507	520
Hb.	1	91	88	88	85	86	80	75	78	83
	2	81	73	73	72	73	66	59	67	75
Hb-index	1	0.70	0.76	0.76	0.85	0.78	0.72	0.67	0.67	0.69
	2	0.70	0.71	0.71	0.82	0.78	0.69	0.62	0.66	0.72
Blood catalase	1	7.11	6.43	6.56	6.53	6.70	6.46	6.60	6.97	7.28
	2	5.92	5.00	5.51	5.34	5.85	4.79	4.86	6.22	6.43
Plasma catalase	1	18.02	17.34	18.02	18.70	23.12	24.82	20.06	17.34	18.02
	2	11.22	8.84	9.52	11.56	18.36	16.32	17.00	8.16	11.56
Catalase index	1	1.09	1.11	1.14	1.29	1.21	1.16	1.17	1.19	1.21
	2	1.02	0.97	1.07	1.22	1.25	1.00	1.02	1.22	1.23
Hb-catalase index	1	0.45	0.43	0.43	0.45	0.45	0.47	0.51	0.52	0.51
	2	0.43	0.40	0.44	0.43	0.46	0.42	0.48	0.54	0.50
Plasma-blood catalase ratio	1	2.54	2.70	2.75	2.87	3.45	3.85	3.04	2.49	2.47
	2	1.90	1.76	1.73	2.17	3.14	3.41	3.50	1.31	1.80

TABLE VI

Blood and Plasma Catalase Content by 2% Japanese Ink Injection

	No.	Before injection	After injection (time in hours)								
			0.5	1	2	3	4	5	6	8	12
Erythrocytes	1	591	560	523	511	530	518	522	540	556	588
	2	620	584	549	530	549	550	582	616	611	623
Hb.	1	80	75	75	71	72	65	60	73	78	81
	2	95	84	80	78	79	75	78	84	88	92
Hb-index	1	0.68	0.67	0.73	0.69	0.68	0.63	0.57	0.68	0.70	0.69
	2	0.77	0.72	0.73	0.74	0.72	0.68	0.67	0.68	0.72	0.74
Blood catalase	1	6.05	5.07	5.13	5.03	5.30	5.85	5.68	6.22	6.49	6.19
	2	6.77	5.44	5.54	5.44	6.15	6.39	6.12	6.90	7.14	6.56
Plasma catalase	1	10.88	9.52	8.84	10.88	17.00	16.33	19.04	12.24	13.60	14.96
	2	11.90	8.84	9.52	12.24	12.92	17.68	19.72	16.32	12.92	13.94
Catalase index	1	1.02	0.90	0.98	0.99	1.00	1.15	1.09	1.15	1.16	1.05
	2	1.09	0.93	1.01	1.03	1.12	1.16	1.05	1.12	1.17	1.05
Hb-catalase index	1	0.44	0.39	0.40	0.41	0.43	0.52	0.55	0.49	0.48	0.44
	2	0.41	0.38	0.40	0.41	0.45	0.50	0.46	0.48	0.47	0.41
Plasma-blood catalase ratio	1	1.80	1.88	1.72	2.16	3.21	2.80	3.35	1.93	2.10	2.41
	2	1.76	1.62	1.72	2.25	2.10	2.77	3.22	2.36	1.81	2.12

TABLE VII

Blood and Plasma Catalase Content by 3% Japanese Ink Injection

	No.	Before injection	1st day		2nd day		3rd day		4th day		5th day		6th day after injection	7th "	8th "	9th "	10th "
			Before injection	1h after injection	Before injection	1h after injection	Before injection	1h after injection	Before injection	1h after injection	Before injection	1h after injection					
Erythrocytes	1	601	631	598	633	625	631	620	595	544	616	600	652	630	642	653	642
	2	700	700	618	653	658	713	683	735	667	664	627	648	655	670	632	622
Hb.	1	88	94	90	86	84	89	85	88	86	92	85	101	100	93	98	94
	2	91	98	91	94	96	102	98	100	105	95	98	95	98	94	100	87
Hb-index	1	0.73	0.75	0.75	0.68	0.67	0.71	0.68	0.74	0.79	0.75	0.71	0.78	0.79	0.72	0.75	0.73
	2	0.65	0.70	0.74	0.72	0.73	0.72	0.72	0.68	0.79	0.72	0.78	0.73	0.75	0.70	0.79	0.70
Blood catalase	1	8.28	8.36	8.33	7.24	7.24	9.11	8.47	8.53	7.68	9.21	8.40	8.40	9.01	8.33	8.23	6.90
	2	6.15	5.61	6.70	7.24	7.45	7.48	6.36	7.21	7.58	6.53	6.36	6.15	6.60	6.19	5.88	7.72
Plasma catalase	1	17.68	14.28	10.88	12.24	8.84	11.22	8.16	9.52	8.16	10.88	5.78	5.10	8.50	10.88	14.96	16.32
	2	13.60	14.96	9.52	10.20	8.16	14.96	7.48	11.56	8.84	12.24	3.40	4.76	10.20	10.88	10.88	8.84
Catalase index	1	1.38	1.32	1.39	1.14	1.16	1.44	1.36	1.43	1.41	1.49	1.40	1.28	1.43	1.30	1.26	1.07
	2	0.88	0.80	1.08	1.11	1.14	1.05	0.93	0.98	1.13	0.98	1.01	0.95	1.00	0.93	0.93	1.24
Hb-catalase index	1	0.55	0.52	0.54	0.51	0.50	0.59	0.58	0.56	0.52	0.58	0.57	0.48	0.52	0.52	0.49	0.43
	2	0.39	0.32	0.43	0.45	0.45	0.43	0.38	0.42	0.42	0.40	0.38	0.38	0.39	0.38	0.34	0.51
Plasma-blood catalase ratio	1	2.13	1.71	1.31	1.69	1.22	1.23	0.97	1.11	1.16	1.18	0.69	0.61	0.94	1.31	1.82	2.36
	2	2.22	2.66	1.42	1.41	1.09	2.00	1.18	1.60	1.17	1.88	0.54	0.77	1.55	1.76	1.85	1.15

TABLE VIII

Blood and Plasma Catalase Content by Colloidal Silver Injection

	No.	Before injection	1st day after injection	3rd " "	5th " "	7th " "	9th " "	11th " "	13th " "	17th " "	21th " "	25th " "	29th " "	35th " "	40th " "	50th " "	60th " "	70th " "
Erythrocytes	1	616	729	468	530	522	516	532	486	507	446	430	458	518	570	540	556	560
	2	618	575	644	682	544	565	540	562	424	520	311	529	527	600	630	650	649
Hb.	1	80	98	63	68	75	65	66	64	55	63	57	63	74	80	80	82	84
	2	85	82	87	80	77	75	68	70	62	58	56	62	71	86	88	98	100
Hb-index	1	0.65	0.67	0.67	0.64	0.68	0.63	0.62	0.66	0.55	0.71	0.66	0.70	0.71	0.74	0.74	0.74	0.75
	2	0.69	0.71	0.68	0.59	0.71	0.65	0.63	0.62	0.73	0.56	0.90	0.59	0.62	0.72	0.70	0.75	0.77
Blood catalase	1	5.68	5.13	3.77	3.98	4.93	4.96	4.83	3.54	5.24	4.96	5.71	5.34	4.79	5.30	4.42	3.33	3.13
	2	5.61	5.51	4.59	5.75	5.51	5.00	4.90	4.69	4.86	5.20	5.30	4.83	5.07	5.61	5.10	3.88	3.94
Plasma catalase	1	47.94	35.36	23.12	31.28	43.52	45.56	58.48	30.60	26.52	53.04	34.00	38.76	18.36	23.12	10.54	6.80	7.48
	2	34.00	23.80	12.24	47.60	51.68	44.88	49.64	39.44	42.84	55.08	34.68	22.44	53.72	23.80	13.60	7.48	10.20
Catalase index	1	0.92	0.70	0.81	0.75	0.89	0.96	0.91	0.74	1.03	1.11	1.33	1.16	0.92	0.93	0.82	0.60	0.56
	2	0.99	0.89	0.71	0.84	1.01	0.89	0.91	0.83	1.14	1.00	1.70	0.91	0.96	0.93	0.81	0.60	0.61
Hb-catalase index	1	0.41	0.30	0.35	0.34	0.38	0.44	0.43	0.32	0.53	0.46	0.58	0.49	0.38	0.38	0.32	0.24	0.22
	2	0.38	0.39	0.31	0.42	0.41	0.39	0.42	0.39	0.45	0.52	0.55	0.45	0.41	0.38	0.34	0.23	0.23
Plasma-blood catalase ratio	1	8.29	6.88	8.64	7.85	8.82	9.17	12.08	8.67	5.07	10.70	5.96	7.24	3.82	4.34	2.38	2.04	2.39
	2	6.06	4.32	2.67	8.28	9.39	8.98	10.11	8.40	8.81	10.57	6.55	4.64	10.71	4.24	2.67	1.93	2.59

1% colloidal silver for 20 days lowered the blood and plasma catalase content, in particular, the decrease of the latter preceded that of the former (Table VIII).

Discussion

At present it is generally recognized that the spleen is one of important hemopoietic organs, but workers differ in opinion as to the function of the spleen. Hirschfeld[3], Roese[4] and Yoshioka[5] report that the spleen represses the hemopoietic function of the bone-marrow, because erythrocytes, especially young cells increase by slpenectomy. On the contrary, Eppinger[6], Istomanowa[7] and Kô[8] assert that the spleen stimulates the function of the marrow, because the decrease of red cells take place by splenectomy. But Pugliese[9] and Gon[10] believe that the spleen is functionless hemopoietically. Moreover, many investigators make objections to the hemopoietic efficiency of the spleen extract. Zih[11], Birke[12], Nipperdey[13] and Imai[14] recognize the decrease of erythrocytes by an injection of the spleen extract, on the contrary, Leake[15] and Kô[8] report their increase.

As for the relation between the spleen and the blood catalase, Winternitz[16], Zatti[17] and Ri[18] recognize that the blood catalase content decreases by splenectomy, but Okunev[19] reports its increase. Moreover, Ri[18] and Kawabata[20] assert that the blood catalase content increases by an injection of the spleen extract, while Ri[18] has not verified such increase in splenectomized rabbits. But there has been no report concerning the relation between the spleen and the plasma catalase content.

Our results obtained in these experiments show that the plasma catalase content decreases remarkably by splenectomy, but erythrocytes, hemoglobin and the blood catalase content are almost unchangeable. As for the influence of the spleen extract injection we find the marked decrease of erythrocytes, hemoglobin and the blood catalase content, in spite of the increase of the plasma catalase in normal rabbits, but such efficiency is not revealed in splenectomized rabbits.

Therefore, the spleen has a close connection with the creation of the plasma catalase, perhaps, because of its hemoclastic function.

As to the influence of the RES upon the hemopoietic function, Müller[21] and Komiya[22] report that the injury of the RES causes anemia. On the contrary, Nissen[23], Aikô[24] and Ukita[25] assert that the stimulation of the RES causes the increase of red cells. But there is no report concerning the influence of the RES upon the blood and plasma catalase content. It is generally accepted that the Japanese ink with high percentage blockades the RES, on the contrary the solution with low percentage stimulates it. Moreover, the RES is stimulated by injection of

the congo-red solution (Becker[26], Behr[27] and Fukuda[28]), but it is blockaded by the injection of the colloidal silver (Yamagata *et al.*[29]).

According to our experiments, it is presumed that the stimulation of the RES causes to increase the plasma catalase content, on the contrary, the blockade of the RES as well as splenectomy causes its decrease.

Thus it can be concluded that the plasma catalase is produced in the RES, especially in the spleen, perhaps because of its hemoclastic function.

Summary

From these experiments it can be concluded as follows: the plasma catalase is produced in the RES, especially in the spleen. That is to say, the elevated function of the RES and the spleen causes to increase the plasma catalase content, but the declined function causes the decrease of the plasma catalase content.

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Studies on Blood and Plasma Catalase

The 6th Report

Influence of the Liver upon the Blood and Plasma Catalase Content

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As the liver has the largest quantity of the catalase among the viscera (Battelli[1], Rosenbaum[2], Ochi[3] and Kamimura[4]), it may be presumed that the liver function has influence upon the blood and plasma catalase content. According to our former report[5], the blood catalase content decreases in liver diseases, but workers differ in opinion as to the relation of the liver function with the blood and palsma catalase content.

Therefore, we attempted to examine the influence of the liver function upon the blood and plasma catalase content and clarify the significance of the glucose therapy combined with vitamin C by a liver injury.

Experimental

Results

A. Influence of the stimulation of the liver function

1. Influence of the intravenous injection of glucose combined with vitamin C or tomato juice

As the control test we confirmed that the intravenous injection of 10 cc. per kg. of 2% sodium chloride solution was uninfluential upon the blood and plasma catalase content (Table I).

Data presented in Table II show that the blood catalase content increased by 10 cc. per kg. of 10 or 25% glucose injection, while erythrocytes, hemoglobin and the plasma catalase content changed in the control test.

Table III shows that the intravenous injection of 100 mg. per kg. of vitamin C (Redoxon) caused a remarkable increase of the blood catalase, while red corpuscles, hemoglobin and the plasma catalase remained unchanged.

Moreover, our results obtained from the combined injection of 10 or 25% glucose with 100 mg. per kg. of Redoxon show that the increase

TABLE I

Control Test by 2% Sodium Chloride Injection

	No.	Before injection	After injection (time in hours)					
			0.5	1	2	3	4	6
Erythrocytes	1	586	618	557	595	586	580	540
	2	548	551	529	540	544	550	539
Hb.	1	86	90	83	83	82	81	80
	2	80	82	78	77	80	80	76
Hb-index	1	0.73	0.73	0.74	0.70	0.70	0.70	0.74
	2	0.73	0.74	0.74	0.71	0.73	0.73	0.71
Blood catalase	1	6.49	6.19	6.15	5.71	5.85	5.95	6.39
	2	5.17	5.10	5.13	5.10	5.03	5.07	5.20
Plasma catalase	1	17.00	14.96	14.28	19.72	17.68	20.40	21.76
	2	10.20	9.52	9.52	10.20	11.22	9.86	10.88
Catalase index	1	1.11	1.00	1.10	0.96	1.00	1.02	1.18
	2	0.94	0.93	0.97	0.94	0.92	0.92	0.96
Hb-catalase index	1	0.44	0.40	0.43	0.40	0.41	0.43	0.46
	2	0.37	0.36	0.38	0.39	0.36	0.37	0.40
Plasma-blood catalase ratio	1	2.62	2.42	2.32	3.45	3.03	3.38	3.18
	2	1.97	1.86	1.85	2.00	2.22	1.94	2.09

TABLE II

Blood and Plasma Catalase Content by 25% Glucose Injection

	No.	Before injection	After injection (time in hours)							
			0.5	1	2	3	4	6	8	12
Erythrocytes	1	418	420	415	403	392	388	398	382	421
	2	460	463	456	449	455	438	451	450	464
Hb.	1	70	71	74	68	68	65	69	70	72
	2	75	77	79	71	70	65	68	66	73
Hb-index	1	0.84	0.85	0.89	0.84	0.87	0.84	0.87	0.92	0.86
	2	0.82	0.83	0.87	0.79	0.77	0.74	0.76	0.73	0.79
Blood catalase	1	6.94	6.90	7.48	7.38	7.34	6.87	6.94	7.00	6.22
	2	5.30	4.69	5.44	6.22	6.12	5.61	5.30	5.03	4.79
Plasma catalase	1	11.22	11.52	13.23	10.27	12.38	12.55	13.02	10.03	10.09
	2	8.16	8.84	9.18	8.50	7.82	9.52	8.16	7.48	7.82
Catalase index	1	1.65	1.65	1.80	1.83	1.87	1.77	1.74	1.83	1.48
	2	1.15	1.01	1.19	1.39	1.35	1.28	1.18	1.12	1.03
Hb-catalase index	1	0.58	0.57	0.59	0.63	0.63	0.61	0.58	0.58	0.50
	2	0.41	0.36	0.40	0.51	0.51	0.50	0.45	0.44	0.38
Plasma-blood catalase ratio	1	1.62	1.67	1.77	1.39	1.69	1.83	1.87	1.43	1.62
	2	1.54	1.88	1.68	1.36	1.27	1.84	1.53	1.49	1.63

of the blood catalase continued for 5 hours after the injection (Table IV).

Instead of vitamin C the combined injection of 10 or 25% glucose with 1 cc. per kg. of tomato juice caused a marked increase of the blood

TABLE III

Blood and Plasma Catalase Content by Vitamin C Injection

	No.	Before injection	After injection (time in hours)							
			0.5	1	2	3	4	6	12	24
Erythrocytes	1	646	640	665	638	636	607	648	640	635
	2	481	460	455	450	478	479	476	480	476
Hb.	1	91	92	101	90	93	92	93	92	90
	2	75	72	70	71	72	70	71	72	72
Hb-index	1	0.71	0.72	0.76	0.71	0.73	0.76	0.72	0.72	0.71
	2	0.78	0.78	0.77	0.79	0.75	0.73	0.75	0.75	0.76
Blood catalase	1	7.82	9.69	10.40	8.98	8.23	7.82	7.11	6.36	6.29
	2	5.20	6.05	5.88	5.81	5.61	4.96	3.91	4.59	5.10
Plasma catalase	1	17.00	20.40	18.36	21.08	13.60	21.76	16.32	14.96	17.00
	2	5.44	4.08	4.08	6.12	6.80	8.50	8.16	6.80	6.12
Catalase index	1	1.21	1.51	1.51	1.40	1.29	1.29	1.10	0.99	0.99
	2	1.08	1.32	1.29	1.29	1.17	1.03	0.82	0.96	1.07
Hb-catalase index	1	0.50	0.61	0.58	0.58	0.51	0.50	0.44	0.40	0.41
	2	0.40	0.49	0.49	0.48	0.45	0.41	0.32	0.37	0.41
Plasma-blood catalase ratio	1	2.18	2.12	1.83	2.34	1.65	2.77	2.30	2.35	2.70
	2	1.05	0.67	0.69	1.05	1.21	1.71	2.09	1.48	1.20

TABLE IV

Blood and Plasma Catalase Content by Combined Injection of 25% Glucose and Vitamin C

	No.	Before injection	After injection (time in hours)					
			1	2	3	4	5	6
Erythrocytes	1	662	658	669	649	653	678	670
	2	539	528	541	538	531	526	530
Hb.	1	93	93	96	94	92	93	95
	2	86	80	87	83	82	82	80
Hb-index	1	0.70	0.71	0.72	0.72	0.70	0.69	0.71
	2	0.80	0.76	0.80	0.77	0.78	0.78	0.76
Blood catalase	1	5.78	5.92	7.04	6.90	6.80	6.19	5.34
	2	4.35	4.86	5.17	4.90	5.51	5.58	5.10
Plasma catalase	1	11.90	10.88	11.22	12.58	13.26	12.92	10.54
	2	11.56	10.88	9.52	12.92	14.96	11.56	12.58
Catalase index	1	0.87	0.90	1.05	1.06	1.04	0.91	0.80
	2	0.81	0.92	0.96	0.91	1.04	1.06	0.96
Hb-catalase index	1	0.36	0.37	0.43	0.43	0.43	0.39	0.33
	2	0.29	0.35	0.34	0.34	0.39	0.39	0.37
Plasma-blood catalase ratio	1	2.06	1.84	1.60	1.82	1.95	2.09	1.98
	2	2.65	2.24	1.85	2.64	2.71	2.07	2.46

catalase content as well as the combined use of glucose and vitaimin C (Table V).

2. Influence of the injection of thyroxin and lecithin

TABLE V

Blood and Plasma Catalase Content by Combined Injection of 25% Glucose and Tomato Juice

	No.	Before injection	After injection (time in hours)					
			1	2	3	4	5	6
Erythrocytes	1	641	682	667	596	638	627	619
	2	607	609	583	591	576	566	570
Hb.	1	88	90	90	96	94	97	96
	2	70	75	74	76	73	75	73
Hb-index	1	0.69	0.66	0.67	0.81	0.74	0.77	0.77
	2	0.57	0.62	0.65	0.64	0.63	0.66	0.64
Blood catalase	1	7.75	9.01	8.47	8.26	8.06	7.96	7.72
	2	7.65	7.82	8.43	8.19	8.13	7.96	7.58
Plasma catalase	1	10.20	10.20	11.22	10.88	14.96	10.88	11.56
	2	19.04	16.32	14.96	12.24	17.00	18.36	17.68
Catalase index	1	1.21	1.32	1.27	1.39	1.26	1.27	1.25
	2	1.26	1.28	1.47	1.38	1.41	1.40	1.33
Hb-catalase index	1	0.51	0.58	0.55	0.50	0.50	0.48	0.47
	2	0.64	0.61	0.66	0.62	0.64	0.62	0.60
Plasma-blood catalase ratio	1	1.31	1.13	1.33	1.32	1.86	1.37	1.50
	2	2.49	2.09	1.78	1.49	2.09	2.31	2.33

TABLE VI

Blood and Plasma Catalase Content by Thyroxin Injection

	No.	Before injection	After injection (time in hours)										
			0.5	1	2	3	4	6	12	24	48	72	96
Erythrocytes	1	532	520	512	498	465	459	407	482	510	512	534	565
	2	475	470	457	456	458	430	485	446	438	528	514	530
Hb.	1	74	72	72	71	70	67	69	75	75	78	76	76
	2	74	72	68	66	63	62	70	72	70	75	73	74
Hb-index	1	0.69	0.69	0.70	0.71	0.75	0.73	0.85	0.78	0.74	0.76	0.71	0.67
	2	0.78	0.77	0.74	0.72	0.69	0.72	0.72	0.81	0.80	0.71	0.71	0.70
Blood catalase	1	5.54	5.95	5.78	5.34	4.93	5.17	5.37	5.98	5.75	7.31	6.63	5.95
	2	5.47	5.58	5.71	5.58	5.44	4.66	4.49	4.73	4.66	5.58	7.21	6.63
Plasma catalase	1	7.48	12.24	12.92	9.52	8.16	7.48	8.16	5.44	9.52	6.80	8.16	9.52
	2	8.84	10.56	12.92	12.24	8.84	8.84	4.76	6.80	9.52	6.80	7.48	5.44
Catalase index	1	1.04	1.14	1.13	1.07	1.06	1.12	1.32	1.24	1.12	1.42	1.24	1.05
	2	1.15	1.18	1.25	1.22	1.19	1.08	0.93	1.06	1.06	1.06	1.40	1.25
Hb-catalase index	1	0.44	0.48	0.47	0.44	0.41	0.45	0.45	0.46	0.44	0.54	0.51	0.40
	2	0.43	0.45	0.49	0.49	0.50	0.43	0.37	0.38	0.39	0.43	0.57	0.52
Plasma-blood catalase ratio	1	1.35	2.06	2.24	1.78	1.65	1.44	1.52	0.91	1.65	0.93	1.23	1.60
	2	1.61	1.89	2.26	2.20	1.62	1.90	1.06	1.44	2.04	1.22	1.04	0.82

As presented in Table VI, the subcutaneous injection of 0.7 cc. per kg. of thyroxin cuased an increase of the plasma catalase content, instead

TABLE VII

Blood and Plasma Catalase Content by 10% Lecithin Injection

	No.	Before injection	After injection (time in hours)								
			1	2	3	5	8	12	24	48	72
Erythrocytes	1	590	576	570	560	553	528	503	481	477	488
	2	499	479	460	381	399	364	376	387	342	359
Hb.	1	86	88	86	83	80	72	66	63	60	62
	2	65	67	63	57	60	56	54	53	50	50
Hb-index	1	0.73	0.76	0.75	0.74	0.72	0.68	0.66	0.65	0.63	0.64
	2	0.65	0.50	0.68	0.75	0.75	0.77	0.72	0.68	0.73	0.70
Blood catalase	1	6.36	6.46	6.19	6.09	6.12	4.79	4.35	4.66	5.07	4.79
	2	4.73	4.56	4.15	3.50	3.50	2.89	2.96	3.16	3.88	3.13
Plasma catalase	1	13.60	14.96	19.72	25.16	33.32	23.12	21.76	22.10	14.96	7.48
	2	8.16	11.56	14.96	34.00	46.92	61.20	31.96	13.94	11.56	7.48
Catalase index	1	1.08	1.12	1.09	1.09	1.11	0.91	0.86	0.97	1.06	0.98
	2	0.95	0.95	0.90	0.92	0.88	0.80	0.79	0.82	1.13	0.87
Hb-catalase index	1	0.43	0.43	0.42	0.43	0.44	0.39	0.38	0.43	0.49	0.45
	2	0.42	0.40	0.38	0.36	0.34	0.30	0.32	0.35	0.45	0.36
Plasma-blood catalase ratio	1	2.14	2.31	3.19	4.13	5.43	4.82	5.00	4.74	2.95	1.56
	2	1.72	2.54	3.61	9.71	13.38	21.18	10.78	4.41	2.99	2.39

TABLE VIII

Blood and Plasma Catalase Content by Chloroform Intoxication

	No.	Before injection	After injection (time in hours)						
			1	3	5	8	12	24	48
Erythrocytes	1	589	578	560	600	608	598	560	542
	2	486	484	460	503	500	498	470	442
Hb.	1	86	83	81	96	98	108	90	83
	2	70	72	71	82	93	98	85	78
Hb-index	1	0.74	0.72	0.72	0.80	0.81	0.90	0.80	0.77
	2	0.72	0.74	0.77	0.82	0.93	0.98	0.90	0.88
Blood catalase	1	6.32	5.85	5.64	5.85	5.95	5.92	5.00	4.35
	2	4.69	4.45	4.52	4.59	4.66	4.28	2.96	2.21
Plasma catalase	1	11.56	30.60	10.88	8.16	15.64	30.60	31.92	22.44
	2	8.84	18.36	8.16	12.92	18.36	29.92	31.92	25.84
Catalase index	1	1.07	1.01	1.01	0.98	0.98	0.99	0.89	0.80
	2	0.97	0.92	0.98	0.91	0.93	0.86	0.63	0.50
Hb-catalase index	1	0.43	0.41	0.41	0.35	0.35	0.32	0.32	0.30
	2	0.39	0.36	0.37	0.33	0.29	0.25	0.20	0.16
Plasma-blood catalase ratio	1	1.83	5.23	1.93	1.39	2.63	5.17	6.30	5.16
	2	1.88	4.12	1.80	2.81	3.94	6.98	10.80	11.68

of the decrease of the blood catalase content which increased for 3 or 4 days after the injection.

The above-mentioned tendency can be made more remarkable by the intravenous injection of 10 cc. per kg. of 10% lecithin solution than

by the injection of thyroxin (Table VII).

B. Influence of the liver injury

1. Influence of chloroform intoxication

Our results obtained by chloroform intoxication in doses of 0.25 cc. per kg. show that the blood catalase content decreased at first slowly and more remarkably 2 or 3 days later, on the contrary, the plasma catalase content increased almost suddenly 8 hours later (Table VIII). On the other hand, the hemoglobin tends to increase, but erythrocytes is almost unchangeable.

2. Influence of phosphor intoxication

Data presented in Table IX show that the blood catalase content decreased remarkably by the subcutaneous injection of 0.75 cc. per kg. of 0.5% phosphor, contrary to the increase of the plsama catalase content, while erythrocytes and hemoglobin remained almost uninfluenced.

3. Influence of glucose-vitamin C by liver injury

When 10 cc. per kg. of 25% glucose combined with 100 mg. per kg. of Redoxon was intravenously injected 8 hours after the above-mentioned chloroform intoxication, the declined content of blood catalase was elevated and the increased content of plasma catalase was repressed for 4 hours, as shown in Table X.

In the similar manner, the combined use of 25% glucose 10 cc. per kg. with Redoxon 100 mg. per kg. checked the decrease of the blood catalase content and the increase of the plasma catalase content in the case of the above-mentioned phosphor intoxication (Table XI).

Discussion

Since Minot and Murphy[6] brought forward liver treatment for pernicious anemia, it has been noticed by many investigators that the liver has a very important hemopoietic function. But there is no concordant opinion as to the relation of the liver function with the blood and plasma catalase content.

It is generally accepted that glucose promotes the liver function by means of the increased liver glycogen content. According to Iizuka[7] and Morita[8], the hypertonic glucose solution causes to increase the blood catalase content, but Giwjorra[9] believes it to be uninfluenced. We[10] have confirmed that glucose, vitamin C and tomato juice promote the function of the liver and the reticulo-endothelial system (RES). Gentile[11] reported that the blood catalase content decreases in the marmot with C-avitaminosis. Moreover, Török and his pupils[12] asserted that the above-mentioned decrease of the blood catalase content is checked by vitamin C injection, which has no effect upon the blood catalase content of normal marmot. Our results obtained in these experiments show

TABLE IX

Blood and Plasma Catalase Content by Phosphor Intoxication

	No.	Before injection	After injection (time in hours)						
			1	3	5	8	12	24	48
Erythrocytes	1	353	322	360	325	360	341	353	378
	2	489	458	484	440	459	487	525	594
Hb.	1	58	51	53	52	50	52	50	48
	2	76	70	64	60	66	70	73	65
Hb-index	1	0.82	0.79	0.74	0.80	0.70	0.76	0.71	0.64
	2	0.78	0.76	0.66	0.68	0.72	0.72	0.69	0.55
Blood catalase	1	8.64	7.00	6.32	6.46	5.75	6.43	5.92	7.21
	2	7.51	7.51	6.53	6.77	7.82	7.24	6.80	6.46
Plasma catalase	1	12.92	16.32	11.56	12.24	35.36	21.08	36.72	57.80
	2	6.80	9.52	11.56	10.88	14.28	21.08	34.68	35.70
Catalase index	1	2.44	2.17	1.76	1.98	1.59	1.88	1.67	1.90
	2	1.53	1.63	1.35	1.53	1.70	1.49	1.29	1.08
Hb-catalase index	1	0.86	0.80	0.69	0.73	0.67	0.72	0.69	0.87
	2	0.52	0.63	0.59	0.66	0.69	0.60	0.54	0.58
Plasma-blood catalase ratio	1	1.95	2.33	1.83	1.90	6.13	3.27	6.21	8.02
	2	0.91	1.27	1.77	1.61	1.83	2.91	5.10	5.52

TABLE X

Blood and Plasma Catalase Content by Combined Injection of 25% Glucose and Vitamin C after Chloroform Intoxication

	No.	Before chloroform intoxication	8h after chloroform intoxication	After glucose and vitamin C injection (time in hours)					
				1	2	3	4	5	7
Erythrocytes	1	458	507	447	448	459	459	470	509
	2	623	587	638	575	516	537	517	526
Hb.	1	75	80	68	71	75	68	63	61
	2	90	90	94	79	78	77	80	86
Hb-index	1	0.82	0.79	0.76	0.79	0.82	0.74	0.67	0.60
	2	0.72	0.76	0.74	0.69	0.76	0.72	0.77	0.82
Blood catalase	1	4.62	4.66	4.90	4.45	4.59	4.73	4.62	4.42
	2	4.56	5.64	5.98	5.00	4.90	4.76	4.83	4.66
Plasma catalase	1	4.76	70.72	29.92	42.84	29.92	27.20	38.08	59.84
	2	7.48	36.04	9.52	10.88	8.84	10.88	35.36	68.68
Catalase index	1	1.01	0.92	1.09	0.99	1.00	1.03	0.98	0.87
	2	0.73	0.96	0.94	0.87	0.95	0.88	0.93	0.89
Hb-catalase index	1	0.36	0.34	0.42	0.37	0.36	0.40	0.43	0.42
	2	0.29	0.36	0.37	0.37	0.37	0.36	0.35	0.33
Plasma-blood catalase ratio	1	1.03	15.13	6.11	9.62	6.51	5.76	8.23	13.52
	2	1.64	6.38	1.59	2.18	1.80	2.29	7.32	14.74

TABLE XI

Blood and Plasma Catalase Content by Combined Injection of 25% Glucose and Vitamin C after Phosphor Intoxication

	No.	Before phosphor intoxication	8h after phosphor intoxication	After glucose and vitamin C injection (time in hours)				
				1	2	3	4	5
Erythrocytes	1	668	697	639	651	622	628	642
	2	489	540	538	497	489	470	469
Hb.	1	85	83	82	81	82	78	76
	2	84	68	70	68	68	60	65
Hb-index	1	0.64	0.60	0.64	0.62	0.66	0.62	0.59
	2	0.86	0.63	0.65	0.68	0.69	0.64	0.69
Blood catalase	1	7.51	7.17	7.41	7.55	6.66	6.77	5.98
	2	7.21	5.34	7.41	6.63	7.00	6.05	6.09
Plasma catalase	1	6.80	10.20	7.48	6.80	8.84	12.92	13.60
	2	8.16	21.76	10.88	10.88	16.32	16.32	24.48
Catalase index	1	1.12	1.03	1.16	1.16	1.07	1.07	0.93
	2	1.47	0.99	1.37	1.33	1.43	1.28	1.29
Hb-catalase index	1	0.51	0.50	0.53	0.54	0.47	0.50	0.41
	2	0.50	0.46	0.62	0.57	0.60	0.59	0.54
Plasma-blood catalase ratio	1	0.91	1.42	1.01	0.90	1.33	1.91	2.27
	2	1.13	4.07	1.47	1.64	2.33	2.69	4.02

that the blood catalase increases by glucose injection, especially combined with vitamin C or tomato juice, while the plasma catalase content remains almost uninfluenced.

Therefore, it may be accepted that the promoted function of the liver causes the increase of the blood catalase content.

According to Iizuka[13], Yoshimatsu[14], Kawabata[15] and Ôtani[16], thyroxin, the so-called stimulant of the liver, causes to increase the blood catalase content. But there is no report concerning the influence of lecithin, which is called the stimulant of the liver by Matsuoka[17] and Fujiwara[18]. Data presented in our experiments show that the injected thyroxin as well as lecithin causes to lower the blood catalase content, instead of the increase of the plasma catalase content, as shown in the former report concerning the relation between the promoted function of the RES and the plasma catalase.

Thus it is presumed that thyroxin and lecithin are not stimulant of the liver, but the stimulant of the RES.

As for the relation of the liver function with the blood and plasma catalase content investigators differ in opinion. Tomioka[19] reported that the blood catalase content increases by chloroform intoxication which destroys mainly peripheral areas of the liver lobules, but decreases by phosphor intoxication which destroys mainly central parts of the liver

lobules. On the contrary, Duncker[20] and Nin[21] contended that the blood catalase content increases by phosphor intoxication. Moreover, Kurokawa[22] asserted that the plasma catalase content increases, liberating from the liver. According to our experiments, it is obvious that the blood catalase content decreases remarkably in case of a liver injury caused by phosphor and chloroform, contrary to the increase of the plasma catalase content. And the combined use of hypertonic glucose solution with vitamin C checks the above-mentioned variation of the blood and plasma catalase by a liver injury.

Therefore, it is presumed that the glucose-vitamin C therapy is very useful in the case of a liver injury.

Summary

From these experiments it can be concluded as follows: the promoted liver function causes to increase the blood catalase content, but a liver injury caused by chloroform and phosphor causes to lower the blood catalase content, contrary to the increase of the plasma catalase content. Moreover, the combined use of hypertonic glucose solution with vitamin C can check the variation of the blood and plasma catalase content by a liver injury.

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The Calomel-Solid Food-Hot Compress Therapy of Bacillary Dysentery

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The therapy of bacillary dysentery, reported in the following pages, consists of three elements, i.e. calomel, solid food and hot compress to the bowels. Neither of the trias is dispensable for a rapid, complete recovery from dysentery.

It is needless to add that complete rest in bed is also highly important, and we achieved this in the present therapy as the consequence of practising the hot compress to the bowels. This matter will be dealt with in a later occasion.

Only two days are enough for the complete disappearance of clinical symptoms as well as of the bacillus from feces, if the Calomel-Solid Food-Hot Compress therapy be conducted exactly in the manner described below, and within a few days after the onset, one can return to daily life. Neglect of any of the trias inevitably aggravates condition and makes progress unfavorable. Cases 3 and 4, quoted below, are illustrative.

Shiga discovered the bacillus in 1897; in the next year the start of the present therapy was initiated by the present writer who had soon a good opportunity to utilize it with some success. A great epidemic (over 100 000 cases with a mortality of 21 per cent) took place in the following year in this country widely, so that the region where the writer was living suffered also much therefrom. Much experience improved the method more and more, and convinced the writer of its usefulness by and by[1]. Truth to tell, on the other hand, however the calomel treatment had been abandoned by and by in a long frist, the following short story will show how.

The first use of calomel against the dysentery can be traced to Annesley[2] in 1829, and it was much used in subtropical zones (Griesinger[3], Kartulis[4][5], *etc.*). In reality it was used with success (Baelz[6], Scheube[7][8] for example). Scheube grouped the treatment into two according to dosages. 0.005–0.05 g. calomel an hour for several hours was used by some internists such

as Eichhorst[9)10)], Roux[11)], Law[12)], Kartulis[4)], Segond[13)], Davidson[14)], Plehn[15)]. The clinicians who gave calomel in large doses can be represented by Baelz[6)] (1875–1898, professor in Tokyo) and Scheube[8)] (1877–1881, professor in Kyoto) who lived in Japan for a long time and treated a great number of cases as professors in Tokyo and Kyoto respectively[16)]. The former dosaged 0.5g. calomel at first, followed 30 g. castor oil, and then 0.5 g. calomel each three hours 3–4 times. The latter 0.3–0.5 g. calomel, followed 1–2 table spoons castor oil each 4–6 hours. Calomel was given in toto 3–4 g., *ad maximum* 9 g. Stomatitis can be prevented from occurring by gargling. Similar dosage was prescribed in the books, *etc.* of Griesinger[3)], Amiel[17)], Nenninger[18)], Jochmann[19)], Hoppe-Seyler[20)], Hashimoto[21)], Inouye[22)], etc.

Some of these authors exercised some caution against an occasion of stomatitis, as above related (Scheube[8)], Nenninger[18)], Jochmann[19)], etc.). While occurrence of such a side-symptoms was neglisible to these clinicians on the one hand, some pharmacologists took it very serious (Penzoldt[23)], Tappeiner)[24)].

Despite of lacking any special method of treating the dysentery the clinicians in the subsequent age pushed to disregard the use of calomel here. Good representatives are Strümpell[25)] and Romberg[26)], namely both the text-books (Strümpell and Mering) have been read very widely for a long period. And in fact, the former wrote simply parenthetically that the large dose therapy of calomel is highly recommended only in south countries, and the latter laid no worth to speak of. Similar tendencies are given in Jürgens[27)], Schwalbe[28)], Krehl[29)], Schittenhelm[30)], Brugsch[31)], Schottmüller[32)], Klemperer[33)], Porges[34)], Ozawa[35)], Mitsuhashi[36)], etc. Absorbents are sometimes recommended[37)]. However, the therapy, in general, be not only harmless, but beneficial in recovery of the health.

The period of calomel therapy, formed by Baelz[6)] and Scheube[8)], was in this country afterwards represented for a while by the text books of Inouye[22)] and Hashimoto[21)], and others*. But it was regrettably soon replaced by that of Strümpell[25)] and Romberg[26)], as in occidental countries. The text books of Strümpell and Mering were widely read in this country, in reality among instructors, doctors and students. The next twenty to thirty years is the period of Kuré[38)] and Nishikawa[39)]. It is still continuing to be so. The opinion of the former is similar to Strümpell[25)], and no word is given in the latter.

The new trend has come out now by the introduction of sulfonamide drugs and antibiotic substances. It will be discussed on comparing it with the outcome of the present treatment at the terminal of the paper.

While the calomel therapy has been thus apparently obsolete in general, my experience of the calomel-solid food-hot compress treatment, extending for half a century and having treated a great number of cases, say thousands, with success, is surely worth while to be made public widely, as I have recently an opportunity to complete the long research by the supplement of bacteriological data. It has favoured us with a clear cut proof.

The Routine of the Calomel-Solid Food-Hot Compress Therapy of Bacillary Dysentery

The treatment consists of three principles; the following paragraphs show how it has been and is to be practized here for many years.

(1) On diagnosing a dysentery case, 1.0–3.0 g. calomel is given soon, but as a rule only once. 0.2–1.0 g. for children. Afterwards stomachica, such as sodium bicarbonate water, bitters, diastase (teaspoonful), biofermin (brewers' yeast) are given from time to time.

If a single dose of calomel be proved to be insufficient for resulting a complete recovery, because of a too late application of calomel therapy under some circumstances, in otherwords, of inadequate treatment in the earlier stage or of lacking the faithful practising any of the other essential, given just below, the medicament must be dosaged in advance, as case 4 illustrates.

(2) Before administrating calomel, let patients take solid food as much as possible; boiled rice for adults (corresponds to bread for Western people), balls of boiled rice for children. Vegetables, pickles in plenty; they are rich in fibrous stuffs. Patients take them willingly or with some reductance; children take commonly balls of boiled rice willingly despite of high temperature of the body.

(3) Simultaneous application of heat to the bowels. The compress of high temperature is applied day and night by means of barley or a block of vegetable product (*konnyaku* in Japanese)†, both in sack and boiled. It is needed to renew every twenty minute in order to keep a certain degree of temperature. First place three sheets of towel doubly folded on the abdomen, then barley or *konnyaku*, in sack, boiled thereupon and covered by a soft woolen cloth, such as frannel. Neither gummy nor non-porous sheet should be applied here. The temperature of the barley or *konnyaku* must be kept about 70°C. or higher, and that between the towels and the skin about 50°C. Of these temperatures, etc. some notice is given in later occasions. When tenesmus disappears and frequency of stools diminishes, another kind of compress, i.e. that with not so high temperature is used in late night, in order to save his nurse from further trouble of renewing the compress so frequently. Patient's sleep must not be disturbed too. This is provided by a pocket-warmer or a hot water china bottle. Both apparatuses are constructed specially for such and similar uses. The heat compress in day time and the warmer at night are to be continued until stools recover normal appearance and qualities.

Bacteriological Investigations

It was done of the stools passed from a patient. Inoculate first the

material on the S.S. agar for isolating pure cultures, next inoculate the colonies under question on the Kligler agar for confirming bacilli. Further the genus is determined by means of biochemical and immunological examinations. In the former Gram, motility, glucose-, lactose-, and mannit-splitting properties (indol- and catalase-reaction), were examined, and in the latter agglutination with polyvalent sera, I, II, III, and then with type sera of Komagome BIII and Komagome BI were tested. This was done according to the guide from the Department of Welfare‡.

The cultivation was carried out in fact of the feces, passed at the first examination and then of the calomel stools. Afterwards materials at each evacuation was examined day by day. Sometimes it was taken out directly from the rectum.

The species of bacilli detected were the genus Komagome BIII, and Komagome BI which was proved in a single case. Typus Komagome BIII corresponds to Shigella Flexneri Type 2, Subtype 2a, and Typus Komagome BI to Shigella Flexneri variant Y.

In passing it may be noted of a wave-like variation, here by way of precaution, in detecting various species of Shigella dysenteriae, occurred from year to year. About ten years following the discovery of Shiga, Shigella dysenteriae was found out in predominance; about 1920, Shiga's bacilli in the half of cases, Komagome type in 20%; about 1910, Komagome type in 40%, Shiga only in 0.2%. And in 1945, Shiga in 60%, Komagome type in 15%. Some authors are of opinion that the high percentage of Shiga bacilli might be due to the return of a great number of soldiers etc. from south tropical and subtropical regions[38)40)41)].

The present researches were done in last year, but the Shiga bacilli never found. The present data deal with the dysentery brought about by the Type Komagome, but my clinical experience extends for 50 years, so that the present view is justificable, I believe, to be applicable to the dysentery by the Shigella dysenteriae too, at least.

Clinical Cases

Case 1. K.I. Female. 56 year. (Typus Komagome BI)

2. July, 1951. At night discomfort, feverish, but no other signs, slept good. Next morning headache, nausea, 38.5°C; no appetite, no food intake. Afternoon passed stools consisting of mucus and blood five times in 2 hours, grisping pain in the abdomen. Examination: Tongue whitish covered, puls 80, tenderness on pressure in left hypogastrium. Urine and blood pressure no abnormity. No appetite, two bowls of rice (each 150 g.) with much cold water taken, but slowly. Immediately 3 g. calomel per os. The hot compress with *konnyaku* applied to the abdomen. Until 23 hr. stools with mucus and blood was passed twice, afterwards a large amount of green colored stools passed. After having

applied the hot compression colics diminished by and by. On 4th July 37°C; small amounts of yellowish stools with mucus and blood twice all the day, but the bacilli was detected. Then routine food with much vegetables was given. On 5th yellow-grey half-solid stools once a day, no bacilli, 36.6°C. Feeling of well-being came back. After having administered calomel, the dysentery bacilli disappeared completely in stools. No return of symptoms and no positive proof of bacilli continued for next 14 days. A complete recovery.

Case 2. S.K. Male. 19 year. (Komagome BIII)

18. Aug. 1951. Examination at 7 p.m. This early morning chills, nausea, 39.8°C. From noon on watery stools, grisping pain in the abdomen, then stools with mucus and blood passed, eight times in toto. A bowl rice was taken with difficulty because of occurrence of nausea, but no vomiting. 2 g. calomel *per os*. Hot compress with *konnyaku* to the abdomen. Until next morning a small quantity of stools with mucus and blood passed seven times. Grisping pain persisted still. 37°C, no nausea, appetite good; on 20. small amount of semi-fluid stools with mucus and blood three times, the bacilli were found. Patient's abdomen skin had ordinary appearance while it appears diffusedly red when heated by means of the hot compress such as *konnyaku* or barley boiled. In fact patient did apply only a charcoal-fire-warmer, much milder than these above-given, during night. It is naturally troublesome to renew the hot compress during night, so frequently as every twenty minutes, but it ought not to be replaced by a milder compress however. He then applied again the *konnyaku* compress throughout. Then no tenesmus any more. On 21. no mucus, no blood in stools, grey yellowish and shaped, three times, but the bacilli were detected. Two days later no bacilli. For following thirteen days bacteriological investigations gave only negative results. This case shows a good example how seriously affects an inadequate application of heat to the bowels the outcome of the therapy. Five days were needed to see complete disappearance of bacilli from feces.

Case 3. J.I. Male, 15 year. (Komagome BIII)

19. Aug. 1951, evening examination. On 17. afternoon colics followed by waterly stools three times, but later better. On 18., played base-ball all the day, etc. On 19. early in the morning suddenly fevered, 39.8°C. Each half an hour to one hour stools with mucus and blood passed; chills, headache. Examination. Well built, well fed. Tongue whitish covered, puls 92. Distinct tenderness on pressure in hypogastrium a little on the left side, else no other symptoms. Appetite not yet lost. Since morning water only was taken. Now two bowls of rice taken with pickles, then 2 g. calomel. Hot compress with *konnyaku* to the left hypogastrium. At night colics noticed but slight. Very small amount of stools consisting of mucus and blood three times. Next day (20. Aug.) early morning (at 5.) a large volume of calomel stools passed, and no more fever (36.9°C), no headache and no chills. Later green, mushy stools with mucus and blood three times a day. The bacilli detected. On 21. stools passed twice, no blood, no mucus and no bacilli. Two days of our calomel-

solid food-hot compress therapy was enough to eradiate the infectious agent, and next fourteen days bacteriological investigations were carried out without positive outcome. The organism and clinical symptoms thus rapidly disappeared, and no recurrence occurred.

Case 4. S.I. Female, 57 year. (Komagome BIII)

27. Aug. 1951, examination, four and half days after onset of disease. On 23. early morning suddenly fevered, 38.3°C., thirst, chills, headache, nausea, waterly stools three times, accompanied by grisping pain in the abdomen. An internist dosaged her sulfathiazole, which resulted in reduction of frequency of stools and disappearance of tenesmus and colics. Fever, headache and poor appetite continued further however, and on 27. stools consisting of mucus and blood passed every half hour. These circumstances made her consult us. 40°C., stools as above, tenesmus, colics, no appetite, thirst. Gave two apples only, no rice, because of her weakness. Fatty woman, systolic pressure 170 mm Hg., heart not enlarged, but looked as certainly enfeebled. 2 g. calomel given. Hot compress with *konnyaku* with cautions. Till next early morning small amount calomel stools with mucus and blood, thrice. While headache, colics, and tenesmus disappeared and body temperature fell to 37.5°C, appetite was still very poor, so that only small amount of fluid food was taken. No stomatitis. She felt decidedly better, said she. On the following days stools with mucus and blood passed several times every day; four times on 28., 29., five on 30, three on 31, *etc. etc.* The bacilli did not yet disappear. On 7. Sept. two bowls of rice were given, since appetite became somewhat better; 2 g. calomel dosaged then. On next day a large amount of calomel stools evacuated. From 9. no mucus, no blood in stools, shaped again, and no bacilli.

A great retard of complete recovery, seen in this case, might be reasonably accounted for by an inadequate or insufficient therapy, conducted by another physicians during first five days of the disease and by non-continuance of solid food in the present calomel-solid food-hot compress therapy conducted on the sixth day of disease.

Case 5. K.S. Male, 4 year. (Komagome BIII)

22. Aug. 1951, 9 a.m. Examination. Early morning, colics, stools consisting of blood and mucus four times. 39.3°C. Since no food was taken this morning, two balls of boiled rice of egg size (each 40 g.) given, 1 g. calomel and hot compress to the bowls followed. Until 15 stools with mucus and blood passed twice, then a voluminous calomel stools passed. 37.4°C. On 23., watery stools with a very little amount of mucus and blood passed twice. The bacilli were demonstrated. On the next day grey mushy stools passed, no mucus, no blood. No bacilli proved. On 25. stools well shaped, yellow-gray coloured. Bacteriological tests, done for following fifteen days, evidenced non-existence of the bacilli; no recurrence.

Case 6. S.W. Male, 3 year. (Komagome BIII)

2. Aug. 1951. Examination at 11. From the night before colics accompa-

nied by watery stools every hour, and then muco-bloody stools. 39.6°C, no good appetite but one ball of boiled rice of hen's egg size taken. Drunk much water. 1 g. calomel per os. Hot compress to the bowels could be well carried out owing to his obedient rest on bed. Till 18. stools with mucus and blood once, and calomel stools twice, passed, but no colics, appetite began to recover, 37.5°C. The bacilli were identified there. On the next day, waterly stools with a very little mucus and blood, no more bacilli detected therein. On 24. stools with a very little mucus and blood passed only once; yellowish, mushy stools passed once on 25th. During observations the stools were always mushy, but this is common with this child in daily life. Bacilli were never detected again (From 23. Aug. to 7. Sept.).

Case 7. T.H. Male, 14 year. (Komagome BIII)

29. Aug. 1951. 6. p.m. Examination. From this early morning fevered, 39.3°C, every hour mucobloody feces passed, colics, headache severe. Two bowls of rice, followed by 2 g. calomel. Hot compress was made by boiled barley. Then soon tenesmus, colics and frequency of defecation went to reduce step by step. Until the next morning watery stools with mucus and blood passed twice. At 9 a large quantity of calomel stools passed. In this the bacilli were detected. Next stools on that day became watery. On 31. yellowish, mushy stools twice; no mucus, no blood, the bacilli were not detectable. In the following thirteen days no bacilli were found.

The observations are epitomized in the following small table:

Briefly summarized: It is a matter of half a day to two days after onset of bacillary dysentery to ask physicians in this country, and when the calomel-solid food-hot compress therapy was conducted properly the outcome was always that two days are wholly enough to see the complete recovery of the disease, that is, to see disappearance of clinical symptoms as well as of the bacilli. In a few days, say commonly three to four days, and in fact even on the next day, though not frequently, one can go to work.

That the earlier a therapy conducted, the better the effect is superfluous to say. In reality in describing the data of Marshall *et al.* on sulfaguanidine thereapy, practized at the earliest opportunity at the fronts, Smyly[42] writes: When given during the first two hours of the disease the results are dramatic. If then such be realized with the calomel-solid food-hot compress therapy what would be worked out?

There were two or three cases, in which the calomel-solid food-hot compress therapy was not properly carried out under some circumstances. They are:

The hot compress to the abdomen was not properly conducted in Case 2. While tenesmus, colics not yet abolish, the hot compress, which is so hot as to irritate the abdomen skin and to cause redness there, was

TABLE I

Cases		1	2	3	4	5	6	7
Name & Sex		K.I. ♀	S.K. ♂	J.I. ♂	S.I. ♀	K.S. ♂	S.W. ♂	T.H. ♂
Age		56	19	15	57	4	3	14
Days from onset to the first medical examination		2 days	one day	3 days	5 days	5 hours	one day	one day
Confirmed bacilli		Komagome BI	Komagome BIII	Komagome BIII	Komagome BIII	Komagome BIII	Komagome BIII	Komagome BIII
Dosage of calomel		3.0 g.	2.0 g	2.0 g.	2.0 g	1.0 g.	1.0 g.	2.0 g.
Frequency of diarrhoea		each one hour	8 times	each half an hour or one hour	each half an hour	4 times	each half an hour	each half an hour or one hour
Character of stools		muco-bloodly	muco-bloody	muco-bloody	muco-bloody	muco-bloody	muco-bloody	muco-bloody
Fever		39.5°C.	39.8°C.	39.8°C.	40.°C.	39.3°C.	39.6°C.	39.3°C.
Reduction of fever		one day	one day	one day	one day	one day	one day	one day
Days, required for disappearance of bacilli		2 days	5 days	2 days	In ten days still bacilli found out	2 days	one day	2 days
Results of culture	In the first medical examination	3. July Komagome BI	18. Aug.* Komagome BIII	19. Aug. Komagome BIII	27. Aug. Komagome BIII	22. Aug. Komagome BIII	22. Aug. Komagome BIII	29. Aug. Komagome BIII
	Calomelstools	3. July (+)	19. Aug. (+)	20. Aug. (+)	28. Aug. (+)	22. Aug. (+)	22. Aug. (+)	30. Aug. (+)
		4. July (+)	20. Aug. (+)	From 21. Aug. to 3. Sept. 12 times cultivation no bacilli	From 29. Aug. to 7. Sept. always found out	23. Aug. (+)	From 23. Aug. to 7. Sept. 10 times cultivation no bacilli	From 31. Aug. to 12. Sept. 11 times cultivation no bacilli
		From 5. July to 18. July, in succession no bacilli	21. Aug.† (+)		On 7. Sept. calomel 2.0 g. administered again	From 24. Aug. to 6. Sept. 8 times cultivation no bacilli		
			22. Aug.† (+)		From 9. Sept. to 18. Sept. no bacilli			
			From 33. Aug. to 4. Sept. 9 times cultivation no bacilli					

* From 18. to 20. warm compress
† Hot compress

replaced during the night by another kind of compress not so hot. The reason why he did so, was the troublesome to renew the compress every twenty minutes in order to maintain the temperature applied. Five days elapsed in this case from calomel to the complete recovery. His failure thus affords an evidence to the reader, of indispensability or high usefulness of the application of proper degree of heating the bowels, one of the trias of the calomel-solid food-hot compress therapy. Moisture acts also beneficial.

The hot compress is usually used as a matter of fact in this country in treating dysentery patients, but without accompanying of calomel and solid diet.

Another essential in the trias was not fulfilled in Case 4. About five days elapsed when she asked me, and meanwhile another therapy was carried out and looked enfeebled despite of her corpulence. The enfeebleness of this old woman made me hesitate to urge her to take boiled rice, so that two apples were given before calomel, and on subsequent days only fluid food. Then recovery took place only slowly, so that dysentery stools and bacilli did not disappear ten days after calomel. Now bowls of boiled rice and calomel were given according to our routine. This brought about then the recovery with the usual course in the present therapy. Two days later the bacilli disappeared in good harmony with the remaining cases.

The diet in the calomel-solid food-hot compress therapy, i.e. that solid food is given from the onset of disease, might strike any reader, as contrary ideas have been over-whelmingly prevailing. Following quotations from some modern text books illustrate this. A book notes: Inanition at the early stage, fluid food until stools recover normal characters. Another: Diet must be such as will leave a minimum of residue in the bowels. The diet during the acute stage of that disease is best limited to clear fluid, warmed and in small quantity. With the return of appetite and feeling of wellbeing a bland low residue diet containing adequate protein and vitamins is given. Etc. Etc.

Be recapitulated the diet in the present therapy:—solid food is given, throughout; schematically spoken, some hundred g. (say three hundred) rice boiled, vegetables boiled, and pickles given first soon when diagnosed as dysentery, followed by calomel and hot compress. Then calomel stools passes commonly in half a day, sometimes earlier sometimes later. Meanwhile boiled rice, pickles may be given, if desired. After calomel stools passed, then similar food given *ad lib.* The patients rest in bed; application of hot compress to the bowels necessitates it.

Then the hot compress should be continued until stools recover normal appearance and qualities, or simply said, formed feces passes. The

stools, evacuated after the calomel stools, have blood, mucus, pus no more, but still fluid, semifluid. One or two days are needed after the calomel stools evacuation to see normal characters of stools. Then one can be free from bed, and furthermore can go to daily life.

If the present therapy be applied properly, there is no need to be afraid of too early return to a normal diet and activity. Half a day to one day elapses from the onset of disease to calomel administration—this time can be abbreviated naturally, the shorter the better; "When given during the first two hours of the disease the results are dramatic" (Smyly) —; from calomel administration to calomel stools from some hours to one day, and then to reappearance of normal stools one to two days; then one can return to one's normal life. Three to four days are enough at most to onset of disease to regain the health.

The time needed for the complete recovery, on the one hand, evaluates various kinds of therapy. A book[38)40)], very widely read in this country, notes it in the edition on 1944, as from one or two weeks to one month and in the edition on 1951 in which sulfonamide drugs and antibiotic principles are referred to, from one or two weeks to one month,—as written in the former editions. Another modern textbook (Cheever[42)] 1951) writes: a week of therapy is sufficient to eradicate most infections; the organism rapidly disappears, but recurrences after a week or ten days are not uncommon§.

These figures seem to point out the superiority of the present therapy among those known and widely used, old and new.

Further, it should be added here that we have had no fatal case in about three hundred patients, treated in recent eight years. Since I am an old practitioner, I feel not qualified myself to complete statistics with exactness; and in fact I have been working in recent years clinically with Dr. Mamoru Kikegawa, my son, and he, who is studying in the Medical Department of the University Hospital, conducted the bacteriological research, and on the other hand, we have been helped in preparing this essay by a kind guidance of Dr. Y. Satake, to whom we are much indebted for it.

Only a few words may be spent further in respect to how the trias acts together to heal the disease. Calomel acts to accelerate the peristalsis soon, when given directly into intestines in rabbits in combination with intestinal content while it does so only very slowly when given alone after washing out the latter[43)]. This may be one of reasons why calomel acts strongly in the present therapy where it is preceded by a large mass solid food. Nevertheless, the idea that calomel acts to desinfect the bacilli, put forward as early as half a century ago by Kartulis, should not be abandoned, I believe, without any direct proof. We have never noticed intoxication of calomel despite of a large dosage. It can be accounted for a short interval of staying in the intestines. It is dosaged only once, and evacuated within some hours to one day *ad maximum*.

The administration of solid food before calomel also effects a rapid transportation of intestinal contents, which acts in turn to guard the organism from untoward side-effects of calomel. In Case 4, when no solid food was given, the bacilli were found for some days after calomel stools, and clinical symptoms also did not entirely disappeared. On the second dose of the drug, preceded by solid food the bacilli was proved disappeared two days later. Its small cost is also an advantage, though never major, to peoples, though not poor.

Both solid food and hot compress also together act to hasten and strengthen intestinal movements at large, and to diminish at the same time fluid quantity in intestines. Fluid food acts in this respect contradictly.

As above given, the hot compress is very useful or rather indispensable as an element of the trias. What a high temperature is suitable for peoples, living under conditions other than ours? For example, adequate temperature of the warm bath among us is definitely different from among Western people. The difference is 5–7°C. or like.

In adapting the method (diet, heat adaptation with moisture) to other peoples, the various procedures inherent to it must be properly modified for convenience sake.

Summary

The Calomel-Solid Food-Hot Compress Therapy of bacillary dysentery, here reported, is based upon the experience of the present writer extending for a long time and consists of three principles, as the title indicates. As soon as a dysentery case is verified, solid food, which is selected from daily diet, is given, and then calomel in a large dose such as 1.0 to 3.0 g. given, at the same time a hot compress is applied to the bowels, commonly on the left hypogastrium. The clinical symptoms soon apt to diminish; half a day to one day calomel stools passed, in which dysentery bacilli is found out. In subsequent one to two days all the symptoms disappear and no bacilli is detected, and stools have a normal appearance. The bacillary dysentery thus can be exterminated commonly within two to three days after the onset, provided the therapy be started as early as half a day to one day after onset. The earlier the better. And the trias of the therapy should be exactly kept. The recovery is so rapid, and no signs of intoxication, such as stomatitis, etc. No mortality in my recent experience extending for eight years at least. I am anxious to acknowledge exact data of recent outcome with sulfonamide and antibiotic drugs.

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* I. and H. pupils of Prof. Baelz. Dr. Nakanishi, another pupil of B. prescribed also 0.5 g. calomel with castard oil 1–3 times per diem in his lecture in Kyoto University.

† Linseed is used in Germany in like cases.

‡ Department of Welfare, Guide to Sanitary Examination (Jap.), 1951 Tokyo, Tab. I.

§ In the General Meeting in this year of the Jap. Ass. for Infectious Disease, Uchiyama reported of the efficiency of antibiotic substances, especially of chloromycetin. While no side effects were noticed there, he came to witness in some patients the reappearance of fever, diarrhoea and even of bacilli, a few days after stopped the dosage. He gave then a warning against production of bacilli-carriers by the use of antibiotic substances, too. (J. Jap. Ass. Infec. Disease, 1952, **25**, 190.)

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Studies on Myoglobinuria

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Since Theorell's successful experiments[1] in crystalization of myoglobin (to be abbreviated hereafter to mb) in 1932, studies on its physicochemical properties have made rapid progress, while studies in its physiological[2] and clinical aspects have as a matter of fact made practically no progress.

I have been engaged for some years past in the study of mb not only in its serological but also in its physiological (Depart. of Forensic Med., Faculty of Medicine, Hokkaido University: Director: Prof. Ueno)[3][4][5] and clinical aspects, and the results obtained I have duly reported successively, which may be summarized as follows.

1) Heme and myoheme which constitute the prosthetic groups of hemoglobin and of mb respectively, are on the whole identical, while their globin fractions (protein fractions) are quite different. 2) Mb and serum contain identical fractions, and so do mb and muscle proteins (myosin and myogen). 3) Mb and cytochrome C are identical in their protein fractions. 4) Part of the globin contained in mb is contained in bile in slight amounts. This fraction I tentatively term "biliglobin." 5) The species-specifity of mb is serologically remarkable. 6) Mb, on being injected into the blood of a living animal, is partly excreted in the urine; and the remainder is dissociated in the body, appearing partly in the bile, and partly in the serum, while the myoheme constitutes a source of bile pigments. If, on the other hand, hemoglobin is added to a similar test, a part of its heme is converted into myoheme. 7) The essential character of traumatic shock (secondary) is such that mb freed from the acute destructive focus of the tissues, produces auto-antibodies by auto-immunization, causing thereby in the body antigen-antibody reactions, and thus the shock belongs to actual anaphylaxis.

The above is a brief summary of the reports which I have thus far made public on mb. Reports intended for this time are those on myoglobinuria, which, I presume, may form a comparatively interesting

subject clinically, and also on the results of my studies with regard to the relation of the said substance to motion and to spasm respectively.

Experimental

I. Motion and Myoglobinuria

It is a well-known fact that muscular motion, and especially an intensive one, is usually followed by the appearance of albuminuria, which, originating from blood plasma-proteins, is considered as a kind of harmless albuminuria. Since there exists some relation between albuminuria and fatigue, its presence is, needless to say, utilized as a criterion by which to determine the degree of fatigue. If mb is to be taken as one of the particular component parts of the muscle tissues, then mb, being freed from the tissues during muscular motion, should be excreted in the urine, thereby causing the necessary appearance of myoglobinuria. To make sure of this fact, I conducted several experiments. Part of the results has already been published by Higashi and me,[6] which I am going to reproduce here together with other results obtained since.

Method

In order to determine whether motion may cause urinary excretion of mb, I experimented in precipitation by the following antisera, using as antigens the specimens of urine collected before and after the physical activity. The antisera used for this purpose were (1) antihuman mb (prepared after Theorell's method, absorbed previously with human serum, its chief reaction after absorption being 10000×2), (2) antihuman serum (2000×16), and (3) antihuman hemoglobin (previously absorbed with human mb, its chief reaction after absorption being 20000×8). In parallel with precipitation, experiments were also made, by way of reference, with regard to urine-Donaggio-reaction, urine-protien reaction (sulfosalicylic acid) and urine-benzidin-reaction.

Results

1) *Results obtained for a group of* 46 *skaters.* Before the start of race, it was only in one case that urinary excretion of mb occurred, while in the others there was no excretion observed. However, after the race there occurred in all the cases (with 4 exceptions) a urinary excretion of mb with more or less variations in amount from individual to individual. Where the excretion was quantitatively high, the urine-protein reaction was found to be positive, a fact which may be interpreted as a natural sequence, seeing that mb is no other than a chromo-protein.

While engaged in the above investigations, I tested at the same time

Donaggio-reaction which is a customary means for measuring the degree of fatigue, and found that in most of the cases D-values were elevated in parallel with the increased excretion of mb. However, it should be noted that while in some of these cases the increased excretion of mb went hand in hand with the elevation of D-values, in others the increased excretion of mb did not necessarily accompany the proportionate elevation of D-values. Furthermore, I should like to add that owing to the existence of some correlation between the excretion of mb and the length of the race-course, there was a tendency, I noticed, for the excretion to be variable, either higher or otherwise, proportionately to the distance to be covered.

2) *Results performed on a group of ten Marathon* 45 *km. runners.* In this group, I noticed that, owing to the great length of the race-course myoglobinuria appeared in all the cases in large amounts, that is to say, before the start of the race all was negative, while after the race there occurred a copious excretion of mb, and that protein-reaction was on the whole uniformly positive, either strongly or weakly in proportion to the excreted urine volume.

In two cases of this group there occurred following the race an excretion of red urine, whose benzidin-reaction was positive and which, on being microscopically examined, was found to contain only a negligible amount of erythrocytes. These urines were entirely negative with antihuman-hemoglobin serum (after absorption with human mb), and reacted most strongly with antihuman-mb serum. From this it is evident that the red substance which was responsible for the positive benzidin-reaction in the urines was in reality mb, but not hemoglobin at all.

I may turn, now, to the consideration of the way how the specimens of urine collected from the runners reacted with antihuman-serum rabbit serum. In all the cases, the urines collected after the race reacted strongly, but when they were tested by anti-human-serum rabbit serum absorbed with human mb, it was only in three cases that any reaction occurred at all, while in all the others there took place no reaction. For the interpretation of these circumstances the following remarks may be made.

Seeing that a normal serum contains an antigen factor identical with mb, (a fact which I discovered some time before[5]), it follows that anti-human-serum rabbit serum contains antibody against the said antigen-factor, and consequently, it is by this antibody that the mb in the urine reacted with the immune serum, but that with the immune serum absorbed with mb the urine does not react because of the absorption and consequent loss of the antibody.

TABLE I

Skater	Age	Sex	Urine Protein		Urine Donaggio r.		Urine Mb		Race
			before	after	before	after	before	after	
N.I.	18	M.	−	−	19	20	0	100×2	Ice-hockey
A.O.	19	M.	−	−			0	10×1	»
O.I.	19	M.	−	−			0	500×2	»
K.M.	18	M.	−	−			0	0	»
S.S.	19	M.	−	−			0	10×1	»
A.Y.	24	M.	−	−			0	10×1	»
I.Y.	24	M.	−	−			0	10×1	»
S.U.	26	M.	−	+			0	100×2	»
N.K.	28	M.	−	−	7	23	0	100×2	»
U.S.	24	M.	−	−	6	19	0	200×2	»
S.S.	24	M.	−	−	5	20	0	500×2	»
U.S.	27	M.	−	−	3	20	0	50×1	»
S.T.	25	M.	−	−	13	17	0	10×1	»
O.Y.	26	M.	−	−	6	12	0	100×2	»
T.N.	25	M.	−	−	6	17	0	10×1	»
T.K.	19	M.	−	−	10	12	0	10×1	»
M.S.	19	M.	−	−	7	13	0	100×2	»
T.K.	21	M.	−	−	14	18	0	0	»
O.I.	19	M.	−	−	6	9	0	200×2	»
O.O.	20	M.	−	−	7	11	0	10×1	»
M.T.	17	M.	−	+	17	17	0	500×2	»
S.T.	19	M.	−	−	8	17	0	0	»
A.B.	18	M.	−	−	12	6	0	500×2	»
O.I.	23	M.	−	−	20	7	0	500×2	»
Y.K.	18	M.	−	−	16	5	0	0	»
N.H.	30	M.	−	−			0	100×2	»
Y.K.	28	M.	−	−			0	500×2	»
K.H	19	M.	−	+	10	10	10×1	500×2	»
I.T.	27	M.	−	−			0	500×2	»
N.S.	20	M.	−	−	5	19	0	50×1	»
M.S.	19	M.	−	−	16	8	0	100×2	»
N.K.	18	M.	−	−	15	3	0	400×2	10000m
N.S.	18	M.	−	−	5	5	0	400×2	10000m
Y.K.	25	M.	−	−	11	21	0	200×2	1500m
A.K.	19	M.	−	−	11	12	0	500×2	5000m
T.A.	18	M.	−	−	4	11	0	100×2	»
K.M.	18	M.	−	−	20	22	0	100×2	»
O.T.	19	M.	−	−	1	4	0	10×1	
K.T.	18	F.	−	−	14	18	0	100×2	
I.T.	19	F.	±	+	10	12	0	500×2	5000m
T.F.	19	F.	−	−	11	14	0	10×1	»
I.U.	20	F.	−	−	11	21	0	10×1	
N.S.	30	M.	−	−			0	100×2	10000m
O.T.	22	M.	−	+			0	1000×2	»
M.K.	23	M.	−	−			0	100×2	»
T.K.	25	M.	−	+			0	500×2	»

TABLE II

Runner	Urige Protein		Urine B. r.		Urine Dona. r.		Anti Mb		Anti S. (1)		Anti S. (2)		Anti Hb	
	b.	a.	b.	a.	b.	a.	b.	a.	b.	a.	b.	a.	b.	a.
N.K.	−	+	−	−	15	18	0	1000×2	0	1000×4	0	0	0	0
T.B.	−	+	−	−	17	23	0	1000×2	0	1000×4	0	0	0	0
S.S.	−	++	−	−	14	21	0	2000×2	0	1000×4	0	100×1	0	0
K.I.	−	++	−	++	19	23	0	5000×2	0	2000×4	0	100×1	0	0
F.I.	−	±	−	−	17	22	0	1000×2	0	1000×4	0	0	0	0
K.O.	−	+	−	−	17	22	0	1000×2	0	1000×4	0	0	0	0
M.I.	−	±	−	−	18	23	0	1000×2	0	1000×4	0	0	0	0
M.K.	−	±	−	−	17	20	0	1000×2	0	1000×4	0	0	0	0
K.O.	−	−	−	−	12	19	0	800×2	0	1000×4	0	0	0	0
N.K.	−	++	−	+	11	18	0	5000×2	0	1000×4	0	100×1	0	0

b.=before. a.=after. B.r.=Benzidin reaction. Dona.r.=Donaggio reaction. Anti Mb=Anti human Mb. Anti S. (1)=Anti human serum. Anti S. (2)=Anti human serum (absorbed with human Mb).

II. Spasm and Myoglobinuria

In cases of spasm, also, there alternately occurs successively for a certain period of time a rapid violent contraction and a subsequent relaxation of the muscles, especially of the musculous skeleti. Does this not indicate the evident occurrence of myoglobinuria in spasm?

Clinically, spasmodic fits due to epilepsy, hysteria or other disorders are occasionally accompanied by a temporary appearance of albuminuria. Is it not possible that such albuminuria is in part myoglobinuria?

Method

In order to examine whether mb or any other substances would be excreted in the urines voided after spasmodic fits, I conducted precipitation experiments by the following antisera, using as antigens the specimens collected before and after their occurrence. The antisera employed in the experiments were rabbit immune sera: antihuman-mb (absorbed with human serum, its chief reaction being 5000×2), and antihuman-serum (absorbed with human mb, its chief reaction being 20000×4). As for the determination of whether mb -antibodies did or did not exist in the patients' sera, I used as antigen 10% human-mb solution and for this purpose were used the specimens of urine that had been collected from schizophrenic patients before and after the onset of electroshock fits.

The reason why I had recourse to this method is because in cases of electroshock fit I could have the advantage, which cannot be had in cases of epilepsy, of collecting urine specimens at regular intervals both

before and after the fits and of detecting precisely, at the same time, the relation of the frequency of fits to its effect on the urines.

Results

With regard to the excretion or non-excretion of myoglobinuria following the spasmodic fits the reader is referred to Table III.

In 10 cases no excretion of myoglobinuria was detected preceding the onset of fits, but after the seizures its excretion was observed for a certain definite period of time in all the cases, where the excretion continued for 8 hours beginning half an hour after the seizure (with one exception where the excretion continued for 20 hours). The volume excreted, it seems, was largest for approximately one hour following the fits.

It was also brought to my notice that the specimens of urine collected by myself failed, with a few exceptions, to react with antihuman-serum sera.

Next I investigated the sort of developments that myoglobinuira may undergo with the increasing frequency of spasmodic fits (Table IV).

My investigations revealed that it was during the second occurrence of fits that mb excretion was quantitatively most significantly high, which, however, with the increasing frequency of fits became of smaller volume and of shorter duration, till after the 5th or 6th attack no mb was excreted. Undoubtedly, these circumstances may be influenced to some extent by the intensity of electroshock, but at all events it is nonetheless certain that the increasing frequency of fits brings about a lower rate of mb excretion.

In connection with the appearance of myoglobinuria following the fits, I made experiments with the same gruop of patients to see whether mb freed from the muscle tissues would cause the production in vivo of auto-antibody, but in none of the cases under review I observed any auto-antibodies produced. Of particular interest is this fact, when compared with what Ueno and I[9] discovered some time ago—that mb of the muscle tissues bruised in traumatic secondary shock obtains auto-antigenicity, producing thereby auto-antibodies.

Discussion and Summary

It was disclosed that following muscular motions, particularly motions of a violent nature or spasmodic fits, there occurs from the excessively hard-strained tissues a flow of mb, which, on being excreted in urine, produces so-called myoglobinuria. The albuminuria that occurs on such occasions seems to have been considered as being due to blood plasma-

TABLE III

Patient	K. O.			T. Y.		
Time \ Examination	Urine protein	Anti human Mb	Anti human serum	Urine protein	Anti human Mb	Anti human serum
Before 30 minute	—	—	—	—	—	—
After fits	—	10×2	—	—	200×2	50×2
1 hour	—	200×2	10×1	—	50×2	—
3 "	—	50×2	—	—	100×2	—
8 "	—	100×2	—	—	50×2	—
20 "	—	—	—	—	—	—
Patient	**T. K.**			**I. G.**		
Before 30 minute	—	—	—	—	—	—
After fits	—	10×1	—	—	100×2	—
1 hour	—	50×2	—	—	100×2	—
3 "	—	100×2	—	—	200×2	—
8 "	—	—	—	—	100×2	—
20 "	—	—	—	—	10×1	—
Patient	**I. T.**			**T. I.**		
Before 30 minuie	—	—	—	—	—	—
After fits	—	50×2	—	—	100×2	—
1 hour	—	100×2	—	—	200×2	—
3 "	—	200×2	—	—	200×2	—
8 "	—	10×1	—	—	10×1	—
30 "	—	—	—	—	—	—
Patient	**K. S.**			**K. J.**		
Before 30 minute	—	—	—	—	—	—
After fits	—	100×2	—	—	200×2	—
1 hour	—	10×1	—	±	500×2	50×2
3 "	—	200×2	20×1	—	200×2	—
8 "	—	200×2	—	—	50×1	—
20 "	—	—	—	—	—	—
Patient	**K. F.**			**K. S.**		
Before 30 minute	—	—	—	—	—	—
After fits	—	100×2	—	—	50×2	—
1 hour	—	200×2	10×1	—	100×2	—
3 "	—	100×2	—	—	50×2	—
8 "	—	—	—	—	50×2	—
20 "	—	—	—	—	—	—

protein, but my experiments showed clearly that it is partly due to the excreted mb.

From the results of my experiments I may conclude that there are two different cases: in one case mb undergoes no change when it is excreted in the urine, while in the other case mb is somewhat changed qualitatively in vivo before it is excreted in the urine.

Reports so far made on myoglobinuria have all of them been based

TABLE IV

Patient	Frequency of fits / Time \ Examination	Urine protein	Anti human Mb	Anti human serum	Mb antibodies	Urine Prot.	Anti human Mb	Anti human serum	Mb antibodies
S.I.	Frequency of fits	1				2			
	Before 30 minute	−	−	−	−	−	−	−	−
	After fits	−	−	−		−	100×2	−	
	1 hour	−	100×2	−		−	100×2	−	
	3 "	−	200×2	−		−	200×2	−	
	5 "	−	−	−		−	100×2	−	
	20 "	−	−	−	−	−	−	−	−
	Frequency of fits	3				4			
	Before 30 minute	−	−	−	−	−	−	−	−
	After fits	−	100×2	−		−	100×2	−	
	1 hour	−	100×2	−		−	10×1	−	
	3 "	−	−	−		−	−	−	
	5 "	−	−	−		−	−	−	
	20 "	−	−	−	−	−	−	−	−
	Frequency of fits	5				6			
	Before 30 minute	−	−	−	−	−	−	−	−
	After fits	−	−	−		−	−	−	
	1 hour	−	−	−					
	3 "	−	−	−		−	−	−	
	5 "	−	−	−		−	−	−	
	20 "	−	−	−	−	−	−	−	−
M.O.	Frequency of fits	1				2			
	Before 30 minute	−	−	−	−	−	−	−	−
	After fits	−	100×2	−		+	500×2	50×1	
	1 hour	−	200×2	−		−	100×2	−	
	3 "	−	−	−		−	100×2	−	
	5 "					−	100×2	−	
	20 "	−	−	−	−	±	400×2	−	−
	Frequency of fits	3				4			
	Before 30 minute	−	10×1	−	−	−	−	−	−
	After fits	±	300×2	−		±	300×2	−	
	1 hour	−	−	−		−	200×2	−	
	3 "	−	−	−		−	100×2	−	
	5 "	−	−	−		−	−	−	
	20 "	−	−	−	−	−	−	−	−
	Frequency of fits	5				6			
	Before 30 minute	−	−	−	−	−	−	−	−
	After fits	−	−	−		−	−	−	
	1 hour	−	100×2	−		−	−	−	
	3 "	−	10×1	−		−	−	−	
	5 "	−	−	−		−	−	−	
	20 "	−	−	−	−	−	−	−	−

Mb antibodies=Mb antibodies in serum.

on its supposed existence proved by means of blood reaction (such as guajac or benzidin-reaction, etc.) or by spectroscopical examinations[2)10)11)12)]. For my own part, however, I pursued serological technique for the detection of myoglobinuria, relying on the consideration that the specifity of mb is serologically remarkable. Thus I believe that I have succeeded in my attempt to establish the actual existence of myoglobinuria.

So-called March-hemoglobinuria, which usually appears after overwork on long march, has hitherto been considered as a urinary excretion of hemoglobin caused by the destruction in vivo of the erythrocytes. But my experiments proved that it is in reality part myoglobinuria, and therefore, I am inclined to assert the existence of motion-myoglobinuria or March-myoglobinuria (Ueno, Ono & Higashi[7)13)]). As a rule, myoglobinuria disappears gradually with the increasing intensity of bodily exercise, and so it is of interest to consider this phenomenon in connection with a result of my experiments—myoglobinuria and its relation to the frequency of spasmodic fits.

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On the Effect of Alternating Currents upon the Spectral Sensitivity of the Human Retina

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Much work has been done by a number of investigators[1)-5)] on the effect of a direct current or electrical polarization upon the photosensitivity of the retina, but we have yet no information available as to whether an alternating current has a similar effect. Motokawa, Iwama and Endo[6)] showed that the light threshold at the fovea and parafovea was slightly lowered during passage of subthreshold rectangular pulses of about 20 cycles per second through the eye. In the present investigation we carried out some similar experiments with sinusoidal alternating currents of varying frequencies and test lights of various wave-lengths and obtained some results worthy of mentioning, which will be described in what follows.

Experimental

Method

All experiments were performed in a dark room after a preliminary dark adaptation of about 20 minutes. An alternating current, whose frequency could be varied between 3 and 120 c.p.s., was applied to the eye through a pair of silver electrodes of 2×1.5 c.m^2 in size, placed one on the forehead slightly above the eyebrow and the other on the homolateral temple of the subject. The contact of the electrodes with skin was secured by the use of electrode paste. The strength of A. C. used was about 10% below the threshold for electrically produced phosphenes. The use of stronger currents was of little advantage for the reasons stated later. The threshold voltages were naturally different according to the frequencies of A.C., but did not exceed several volts.

A circular disc of ground glass of 1° in visual angle illuminated from behind with spectral light from a spectroscope served as a target. The patch was always fixated centrally by the left eye, where a minute point of red light served as a fixation mark. The wave-lengths of spectral

lights were calibrated in comparison with the line spectra from a sodium and a cadmium lamp. As a light source for the spectroscope an automobile headlight lamp was used.

The intensity of spectral light was roughly adjusted to the light threshold by controlling the width of the entrance slit of the collimator. For finer adjustment, however, the current strength of the light source was varied by means of a smoothly movable rheostat. No artificial pupil was used, for it was confirmed in a preliminary experiment that such weak A.C. as used in the present investigation had no effect whatsoever upon the pupillar size. Each procedure of experimentation will be mentioned together with the result.

Results

In the first series of experiments the effect of A. C. upon the sensitivity of the retina to spectral lights of certain wave-lengths was tested by the method of "seeing-frequency"[7]. The eye was exposed to light of a certain intensity for a period of 0.5 seconds and the observer was asked to report whether he saw it or not. The intensities were presented in a random sequence, each for a specific number of times, usually 20.

The data obtained from 3 subjects are shown diagrammatically in Fig. 1 (see curves represented by solid circles). The spectral light used in this experiment was orange light of 610 mμ. A series of similar experiments was carried out during application of an A. C. of 62.5 c.p.s. The light stimulus was delivered about 1.5 seconds after the onset of the A. C., which was removed immediately after each subject's report on "seeing." The data obtained in this way are represented by open circles in Fig. 1. As can be seen in this figure, remarkable increases in the seeing-frequency were brought about by the subthreshold A. C. in the subject A, while no such conspicuous effect could be observed in the other subjects, B and C. It is to be noted that there is a striking difference between the curve of A and those of B and C especially in the slope of curves.

In subject C, the response was always of all-or-none type in such a manner that the seeing-frequency was either 100 or 0, never taking intermediate values. On the contrary, the range of intensities for submaximal seeing-frequencies was sufficiently wide in A. The data obtained from B represent an intermediate type between A and C, for submaximal values did appear, but over a much narrower range of intensities than in A. It seems that the type of response stated above has a fundamental significance for the effect of A. C. under consideration, because it showed itself very pronouncedly in A and could be proved only statistically in B, but never in C. The statistical measurements were carried out to make clearer whether or not any effect beyond the limit of experimental

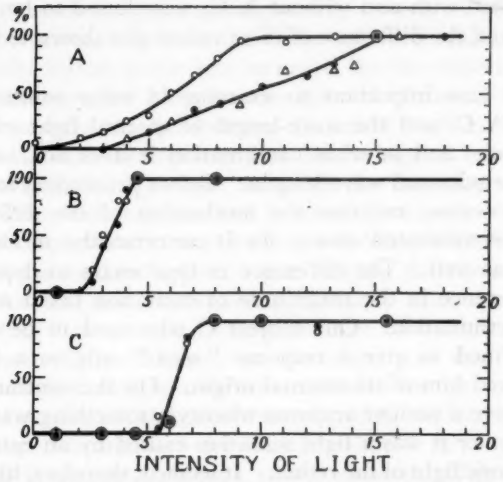

Fig. 1. Relation between the intensity of test light of 610 mμ and the percentage frequency with which the light was seen by 3 observers. Each point represents 20 trials. Points marked by open circles, empty triangles and solid circles were determined with A.C. of 62.5 c.p.s., with A.C. of 42 c.p.s. and without A.C. respectively.

errors could be observed in the subjects B and C.

The intensity of the orange light was fixed at a level roughly corresponding to 50% of seeing-frequency, and thus 10 sequences of measurements, each consisting of 20 trials with A. C. and as many sequences without A. C. were performed, trials with and without A. C. being made in a random pattern. In subject B the average of seeing-frequencies was found 74 for the measurements with A. C. and 53 for the control. The difference of these values was shown to the significant at a 1% level by the test using "student's t." However, no statistically significant difference could be obtained in C. Thus it has been proved in 2 of our 3 subjects that the A. C. of 62.5 c.p.s. enhances the sensitivity of the fovea to the light of 610 mμ.

Next, similar measurements were carried out in subjects A and B applying an A. C. of 42 c.p.s. instead of the A. C. of 62.5 c.p.s., but otherwise under the same experimental conditions as above. The data obtained from A are represented by empty triangles in Fig. 1 A. It can be seen in this figure how exactly the seeing-frequency curve obtained with the A. C. of 42 c.p.s. coincides with the control curve determined without electrical stimulation. The data from B were treated statistically in the same manner as stated above; the average values of seeing-frequen-

cy as determined with and without A. C. were found to be 60.7 and 61.7 respectively, and the difference of these values was shown to be statistically insignificant.

It seems now important to examine in what combination of the frequency of A. C. and the wave-length of spectral light enhancement of sensitivity results and in what combination it does not, over the entire range of frequencies and wave-lengths. Before proceeding to this question, we should, however, mention the mechanism of the different types of response as demonstrated above, for it concerns the mechanism of our phenomenon as well. The difference in type seems to depend primarily upon the difference in the magnitude of excitation taken as the index of threshold determination. Our subject C who used to be of all-or-none type was inclined to give a response "seen" only to a light stimulus which convinced him of its external origin. On the contrary, the subject A seemed to give a positive response whenever something was seen without regard to whether it was a light sensation caused by an external stimulus or due to intrinsic light of the retina. It seemed, therefore, highly probable that the subject C took much greater excitation as the index than did the subject A. To be sure of this relation, we previously instructed the subject A to work with a much clearer index, for example, taking the appearance of the contour of the target as the index, and carried out some experiments. The seeing-frequency curve obtained in this way was found to be as steep as that obtained from subject C, and no effect of A. C. could be observed under this experimental condition.

Hecht, Shlaer and Pirenne[8] assumed the seeing-frequency to be proportional to the probability that a certain critical number of quanta n or more will be delivered to the retina in a given flash, and reached the conclusion that the seeing-frequency curve should become steeper as n increases. As a matter of fact, the general feature of our finding that the curve became steeper when determined with a much greater index, seems to be in agreement with the interpretation by Hecht *et al.*

The intensity range for submaximal seeing-frequencies in the curve A extends over about 0.7 logarithmic units, and this is of the same order as in the 3 subjects of the similar experiment by Hecht *et al.* and in one subject of Crozier's experiment.[9] Therefore, it is likely that the criterion of threshold determination in our subject A was comparable to those of the American observers mentioned above. It was on this subject that we performed further extensive investigations.

Since the number of combinations of frequencies of A. C. and wave-lengths of lights was too great to work with the seeing-frequency method, we had to resort to the usual method of threshold determination. The procedure taken in further experiments was as follows. The observer

raised the current of the light source steadily from a certain subthreshold level till he perceived a light at the center of the fovea, and the current strength at this critical point was recorded by the experimenter. Such a procedure was repeated 8 times at an interval of about 10 seconds. So as to take values only in a steady state, the initial 3 values were discarded, and the light intensity corresponding to the average of 5 values was taken as the light threshold. The rate at which the intensity was raised was such that the threshold was reached in about 2 seconds. In experiments with A. C. the experimenter switched on a subthreshold A. C. of a given frequency as soon as the observer began to raise the current strength for the light source and switched it off immediately after the observer's report on "seeing" was over. The procedure of switching on and off could be done entirely unknown to the subject, so that the possibility of any psychological interferance was excluded in our experiment.

In some experiments the procedure of raising the current was carried out by the experimenter lest the observer should rely upon his tactile sense involved in the manipulation of the rheostat. The data obtained in these experiments were, however, little different from those obtained in the usual manner.

As will be shown below, the degree of sensitivity enhancement due to the action of A. C. was found somewhat greater in these experiments than in those carried out with the seeing-frequency method; under the most favorable conditions the spectral sensitivity was found several times as high as the control sensitivity as determined without A. C. Such a remarkable enhancement must partly be due to some cumulative after-effect, for the enhancement became stronger to some extent as the interval between successive trials was made shorter.

In one occasion the experimenter intentionally interrupted the beam from the light source in one of the successive trials with A. C. Nevertheless the observer gave a response "seen." Even when the interruption of the beam was made known to the observer, he was inclined to give a positive response. However, such a positive response could never be obtained, either in an isolated trial with A. C. or in measurements without A. C. or in successive trials with A. C. of not optimal frequencies. These findings indicate that the positive response to the nil light stimulus stated above can be assigned neither to an illusion nor to a mistake; it must be due to an after-effect of preceding illuminations. As was shown in a previous paper by Motokawa,[10] the electrical sensitivity of the retina is increased by weak light adaptation. In consequence, a subthreshold A. C. alone can elicit a light sensation just localized at the retinal area previously illuminated, especially when the frequency of the A. C. applied is optimal.

In Fig. 2 the sensitivity to spectral lights of various wave-lengths is represented as a function of the frequency of A. C. For test lights of 650 and 610 mμ enhancement of sensitivity occurs over 3 frequency ranges around 77, 62.5 and 52.5 c.p.s. The most prominent maximum is the one situated at 77 c.p.s. in the case of red light and the one at 62.5 c.p.s. in the case of orange light. In the experiment with yellow light of 575 mμ the elevation at 52.5 c.p.s. is most conspicuous. For green light of 515 mμ there can be seen 3 peaks at 52.5, 42.5 and 35 c.p.s., among which the one situated at 42.5 c.p.s. seems to be most prominent. The optimal frequency for the blue receptor must be 35 c.p.s., because the curve for blue light of 465 mμ shows the highest peak at this frequency.

The curve for violet light of 415 mμ has 4 maxima, among which the one situated at 27.5 c.p.s. seems to be most prominent. From these facts it may be inferred that the dominant peaks situated at 77, 62.5, 52.5, 42.5, 35 and 27.5 c.p.s. indicate characteristics of the retinal mechanisms concerned with reception of red, orange, yellow, green, blue and violet lights respectively.

In the next series of experiments, the wave-lengths of spectral lights were varied with fixed frequencies of A. C. The data are represented in Fig. 3. It can be seen in this figure how the range of wave-lengths over which maxima of sensitivity appear is displaced from the violet end of the spectrum toward the red end as the frequency of A. C. is raised. The peaks of sensitivity are seen at 6 definite parts of the spectrum, namely

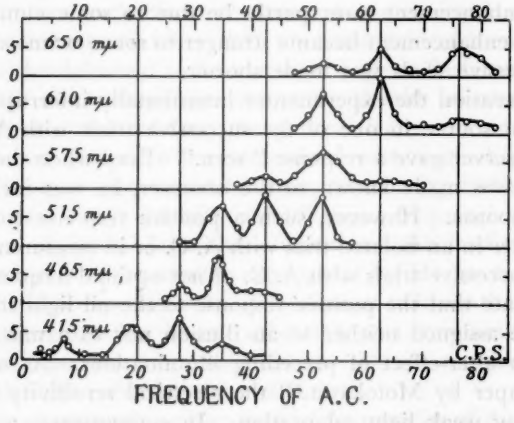

Fig. 2. Effects of subthreshold A.C. of varying frequencies upon the light sensitivity of human fovea. Ordinates: light sensitivity under influence of A.C. expressed in terms of control sensitivity as measured without A.C. Wave-length of test light is given for each curve.

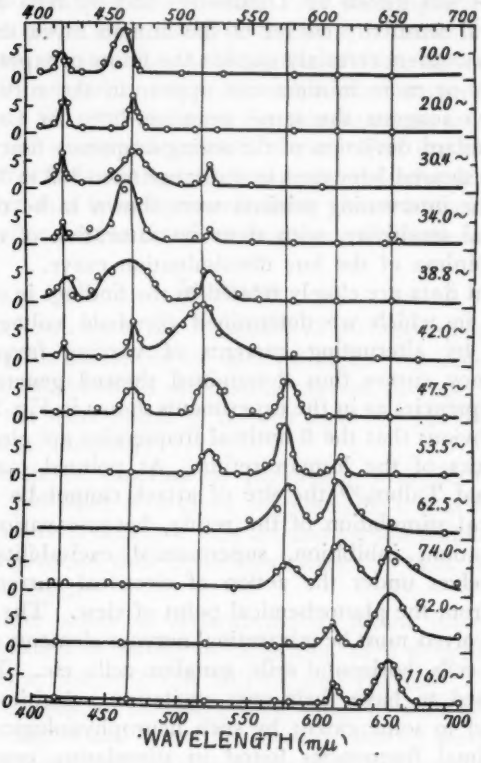

Fig. 3. Spectral sensitivity curves at human fovea during passage of subthreshold A.C. through the eye. Sensitivity is expressed in terms of control sensitivity as measured without A.C. Frequency of A.C. in c.p.s. is given for each curve.

at 650, 610, 575, 520, 465 and 415 mμ. Thus we have obtained 6 different spectral response curves from the human fovea. Since it is a usual manner to characterize retinal elements such as Granit's dominators and modulators and various visual substances by their specific spectral response curves, it would not be too far-reaching to conclude from our findings that 6 kinds of receptor mechanisms have been isolated from the human fovea.

Discussion

We have a number of evidences that retinal mechanisms for color reception are in reality much more complicated than would correspond to such simple schemes as those advocated by some classical color-theories.

For example, it was shown by Thomson[11] and by Hsia and Graham[12] that the spectral sensitivity curves of the human fovea show a number of humps and shoulders certainly outside the limits of experimental errors. The fact that 4 or more minima can appear in the curves for hue discrimination also suggests the same situation.[13][14] In Crozier's experiments[9] the standard deviation of the seeing-frequency function as related to wave-lengths showed 5 maxima in the range from 383 to 712 mμ. These maxima and the intervening minima were shown to be correlated with data on spectral sensitivity, with those on saturation of color and with maxima and minima of the hue discrimination curve.

The present data are closely related to the findings in our most recent experiments,[16] in which we determined threshold voltages for electric flickers caused by alternating currents of varying frequencies. The strength-frequency curves thus determined showed generally 8 minima at the same frequencies as in the experiments shown in Fig. 2 of the present paper. It is obvious that the 8 optimal frequencies are electrophysiological characteristics of the human retina. As pointed out by Granit,[3] Motoakwa[10] and Talbot,[15] the site of attack cannot be photorecipient cells in electrical stimulation of the retina, because various phenomena such as facilitation, inhibition, supernormal excitability, etc. which manifest themselves under the action of electrical currents, cannot be accounted for from the photochemical point of view. The most probable mechanisms involved must be intraretinal nervous elements such as bipolar cells, amacrine cells, horizontal cells, ganglion cells, etc. These elements may be supposed to have their own excitation velocities which would be characterized to some extent by such neurophysiological measures as chronaxie, optimal frequencies found in stimulating experiments with A. C., accommodation constants, etc.

In this connection it deserves attention that in our experiments several optimal frequencies have been found in the human retina. These optimal frequencies are all the more physiologically significant, because it has been shown that the retinal mechanisms characterized by different optimal frequencies are concerned with reception of different colors.

Expressed in physiological terms, our phenomenon represents summation of the effects of both adequate and non-adequate stimuli. It is highly probable that such summation takes place only when photochemically initiated electrical changes impinge upon one and the same neuron as is activated by an electrical stimulus. If the combination of the wave-length of the test light and the frequency of the A. C. applied is not adequate, no summation will result probably because of insufficient overlap of the action fields of the two stimuli. When the electric current is too strong the nervous elements attacked by it will fall into a refractory

state, so that no summation will result. As Sherrington[17] pointed out, summation takes place most easily in the field of neurons which are receiving subliminal excitation. As a matter of fact, no enhancement of photic excitability ensued in our experiments when too strong alternating currents were used for conditioning. For this reason we used subthreshold currents in all the experiments stated above. The use of such a weak conditioning current has another advantage to raise selectivity of its action in such a manner that it activates selectively the neurons having optimal frequencies equal or next to the given frequency of the stimulating current.

Summary

1. It was shown with the "method of seeing-frequency" that the sensitivity of the human fovea to spectral light was enhanced during the passage of a subthreshold alternating current through the eye, if the combination of the wave-length of the test light and the frequency of the A. C. applied was adequate.

2. In a series of experiments performed with A. C. of varying frequencies and test light of a given wave-length, it was found that enhancement of sensitivity occurred only around certain definite frequencies. The optimal frequencies thus established were 77, 62.5, 52.5, 42.5, 35, 27.5, 20, and 7.5 c.p.s.

3. In another series of experiments in which wave-lengths were varied, sharp maxima of sensitivity appeared at 6 definite parts of the spectrum, namely at 650, 610, 575, 520, 465 and 415 mμ. The frequencies of A. C. most adequate for revealing these maxima were higher for those at longer wave-lengths and lower for those at shorter wave-lengths.

4. The phenomenon under consideration was interpreted as due to summation of photochemically initiated and electrically evoked electrical changes at the level of intraretinal neurons.

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Growth Ability of Many Kinds of Actinomyces on the Streptomycin Containing Agar, with Reference to the Changes after Transferring on this Agar

(Studies on the Antibiotic Substances from Aztinomyzes, 8th Report)

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The author has been much interested in studying the growth ability of many actinomyces on the streptomycin-containing agar in the respects, that a strain, which produces an antibiotic substance, may not be much affected by the same compound in its metabolic courses and therefore this procedure will be a suitable tool for the isolation of streptomycin producing strains as stated already by Waksman.[1] Moreover, what kinds of changes of biological properties will be caused by the transmission of streptomyces in streptomycin containing culture media, since many susceptible organisms tend easily to acquire a resistance to this drug in vitro? With special reference to these points, the author made some experiments and obtained several interesting results to be described below.

Experimental

Growth Ability on Streptomycin Containing Agar

Among 258 strains of our laboratory collection, most of which have any antagonistic actions, 52 strains (20.2%) could grow well on Krainsky's agar containing 10 u/cc streptomycin, and when a dosis of 100 u/cc was used, 17 of 52 strains (6.6%) could grow.

When 10 u/cc streptomycin containing agar was used, not only streptomycin producing strains but also other antibiotic and non-antibiotic actinomyces were able to grow on them. Such cases were often met also in other antibiotics containing agar, for example, when chloromycetin containing agar was used to isolate a new chloromycetin-producing strain from soil, streptomycin-producing strains showed also a good growth on this agar.

These facts suggested, that the resistance to an antibiotic was not

strictly specific, especially in the use of a small amount.

However, when a dosis of 100 u/cc streptomycin containing agar was used, among 17 streptomyces strains were able to grow 3 streptomycin producing strains and other 3 strains, which produce an apparently different antibiotic from streptomycin. One of latter 3 strains, K-300 produces a new specific antibiotic and is now under investigation.

The other two strains, K-374 and K-21–25, chromogenic and resembling *Str. roseochromogenus*, showed a streptothricin-like antibiotic spectrum when tested by the streak plate method (so-called "primary screening" in our laboratory).[2)3)] When these culture filtrates, however, were tested by the cup assay method (so-called "secondary screening"),[3)] they showed a spectrum chracteristic of streptomycin. Though they are now under investigation, these strains appear probably to produce both streptomycin and other kinds of antibiotics.

In this respect, the agar containing 100 u/cc streptomycin seems to be so specific that streptomycin-producing strains can grow invariably on it, even if other strains were capable to grow sometimes and this fact was also ascertained with the streptomycin-producing strains newly isolated in our laboratory.

Successive Transmission on Streptomycin Containing Agar

Above mentioned 52 strains of streptomyces were successively transferred on the agar containing gradually increasing amount of steptomycin, 35 of which starting from 10 u/cc and other 17 from 100 u/cc. As shown in Table I, a and b, besides 6 strains mentioned above many other antagonistic and non-antagonistic streptomyces, became able to grow abundantly on the agar containing 50–800 u/cc of streptomycin after such a treatment. However, there were noticed neither morphological nor biochemical changes as well as no remarkable tendency to produce streptomycin itself. Representative example was shown in Table II.

Among several non-antagonistic strains, however, it was able to obtain some slightly antagonistic colonies after transferring to streptomycin containing agar, as Kelner[4)] showed after the treatment by X ray or ultraviolet light. Although these colonies were not streptomycin producing ones, they were characteristic and somewhat resembling these in their specific inhibitory action on *B. anthracis*, because according to our experimental results almost all low antagonistic strains were more highly inhibitory to *B. subtilis* with the exception of streptomycin-producing strains, which were found more strongly inhibitory to *B. anthracis* than *B. subtilis*.

TABLE I

Successive Transmission of Streptomyces Strain on the Streptomycin Containing Agar

Streptomyces group	The highest concentration of streptomycin to which the strain became resistant (u/cc)						Total number
	a. Streptomyces strain capable to grow on 10 u/cc streptomycin containing agar						
	10	50	100	200	400	800	
Ist group, streptomycin producing strains	1	1	1	0	0	0	3
Other strains	1	0	4	1	3	12	21
IInd group	1	0	0	0	3	1	5
IIIrd group	0	0	0	0	0	0	0
IVth group	0	1	0	0	0	0	1
Vth group	0	1	0	0	3	1	5
Total number	3	3	5	1	9	14	

	b. Streptomyces strain capable to grow on 100 u/cc streptomycin containing agar				
	100	200	400	800	
Ist group, streptomycin producing strain	0	2 (342, S-39)	0	1 (346)	3
Other strains	0	0	1 (K-374)	2 (K-300, K-21-25)	3
IInd group	0	1	1	1	3
IIIrd group	0	0	0	2	2
IVth group	0	1	0	0	1
Vth group	0	1	1	3	5
Total number	0	5	3	9	17

Ist group Inhibitory to gram positive, negative and acid-fast organisms.
IInd group Inhibitory to gram positive and acid-fast organisms.
IIIrd grup Inhibitory to gram positive organisms only.
IVth group Inhibitory to acid-fast bacteria only.
Vth group No inhibitory action.

Transmission of Streptomycin Producing Strains

Streptomycin producing strains were also successively transferred through the streptomycin containing agars. The experiments resulted in the facts (a) that the higher the streptomycin concentration of the agar, the fewer the growing resistant colonies, (b) that these resistant colonies were homogeneous in their morphological properties and (c)

TABLE II

Comparison Between Original and Streptomycin Resistant Strain of No. K-374, in Primary and Secondary Screening.

Test bacteria	Primary screening*		Secondary screening†	
	O‡	R§	0	R
Sta. aureus (F.D.A. 209 P)	16.7mm.	21.5	1.9	4.0
Sta. aureus (Terashima)	17.9	23.7	4.0	4.7
B. anthracis	17.6	21.5	4.5	5.2
B. subtilis	16.0	17.0	0.5	1.9
E. coli	11.9	13.0	0.3	1.3
SM-R- *E. coli*	10.5	21.0	0	0
B. agri	7.7	5.2	0	0
O-36-R- *E. coli*	0	0	0	0
Broth Units			13.8 u/cc	19.6 u/cc

* Antibacterial spectrum around the growing streptomyces colonies. The length of inhibition zones were measured in mm.

† Antibacterial spectrum produced by culture filtrate of streptomyces strains. (Cup assay)

‡ Original strain.

§ Resistant strain.

that the yield of streptomycin were at least partially increased than that of original strains in culture broth.

For industrial purpose such a simple procedure seems to be very advisable, in the respect that the transferring cultivation on streptomycin-containing agar is useful at least to prevent "running down" of the potency of streptomycin production and to select any powerful colonies.

Though the results thus far obtained are not yet very efficient, relatively powerful colonies were selected by this procedure and such a method would be also useful for obtaining a limited survival rate.

SUMMARY

1. Several strains of antagonistic and non-antagonistic streptomyces were tested upon their abilities to grow on the streptomycin containing agar. Beside streptomycin producing ones, many strains were able to grow on the agar containing 10 u/cc streptomycin, while only a limited number of streptomyces strains including streptomycin producing ones were capable to grow on the agar containing 100 u/cc of streptomycin. Such a concentration of streptomycin, therefore, appeared to be specific and suitable for isolation and preservation of streptomycin producing strains.

2. Even if the streptomyces strains producing other kind of antibiotics than strptomycin became to grow luxuriantly on streptomycin containing agar, there was seen no tendency to produce streptomycin.

3. It was able to select some, even if weakly, antibiotic colonies from the non-antagonistic strans of streptomyces by serial passages through streptomycin containing agars, although it was impossible to decide whether they were truely streptomycin producing ones, or not. The antibiotic strains thus obtained inhibited *B. anthracis* or *Myco. phlei* more strongly.

4. It was confirmed, that the streptomycin producing strains were reduced in colony numbers, became homogeneous in morphological properties and partially increased in inhibitory activities of culture filtrates, when they were cultivated on streptomycin containing agar through several generations.

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